

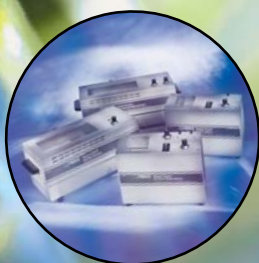
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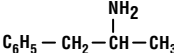
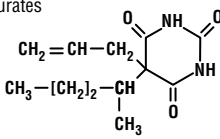
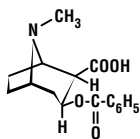
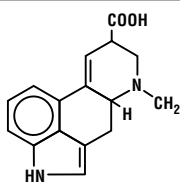
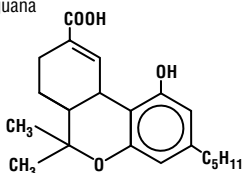
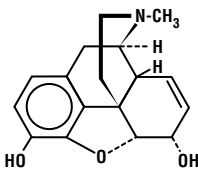
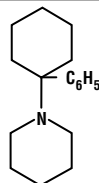
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# Derivatization Reagents for Drugs-of-Abuse

Drug	Form	Reagent	References
Amphetamines 	Amphetamines	BSTFA	1
	Amphetamines	HFBA	2-5
	Amphetamines	HFBA/PFPA	6
	Amphetamines	MSTFA with TMCS	7
	Amphetamines	TFAA	7,8
	Methamphetamine	TFAA	9,10
Barbiturates 		BSTFA	1
		MethElite™ Reagent	11-13
		Methyl-8® Reagent	14,15
		PFBBr	16
Cocaine 	Benzoylcocgonine	BSTFA/Butyl Iodine/TMAH	17
		BSTFA	1,18
		MTBSTFA	19
		PFPA/PFPOH	9,20
LSD 		BSA	21
		BSTFA	22
		MSTFA	21
		TFAI	23
Marijuana 	THC metabolites	BSA	24
		BSTFA/BSTFA+1% TMCS	24-27
		BSTFA/TMCS/TMSI	24
		MSTFA	9
		MSTFA/MSTFA+1% TMCS	27
		MTBSTFA	28
		PFBBr	29
		PFPA/HFIOH	30
		PFPA/PFPOH	31
		TFAA & BF <sub>3</sub> /MeOH	32
		TMPAH	9
	TMSI	24	
Opiates 	Morphine	BSTFA+1% TMCS	33
		MBTFA	34
		PFPA	35
		TFAA	36
	Morphine/Codeine	BSTFA	1,37
		BSTFA+1% TMCS	38,39
		BSTFA/TFAI	40
		HFBA	38
		MBTFA	38
		PFPA	38,41
		PFPA/HFBA	37
		PFPA/PFPOH	9
		TFAA	42
		Trimethylsilyl	43
PCP 	PPC/PCHP/PCP	BSTFA+1% TMCS	44
		HFBA	45

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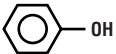
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# Derivatization Reagents for Specific Functional Groups

Functional Group	Procedure	Reagent	Derivative	Notes		
<b>Amides</b> $\begin{array}{c} \text{O} \\    \\ -\text{C}-\text{NH}_2 \\ \text{Primary} \end{array}$	Silylation	BSA	TMS Amides	Difficult to form due to steric hindrance		
		BSTFA	TMS Amides			
		BSTFA+TMCS	TMS Amides		TMCS used as a catalyst	
		MSTFA	TMS Amides		Reaction byproducts more volatile	
		MSTFA+TMCS	TMS Amides			
	Secondary	Silylation	Tri-Sil <sup>®</sup> Reagents	TMS Amides	Difficult to form; very stable TBMCS aids derivatization	
			MTBSTFA	TBMCS Amides		
		MTBSTFA+TBMCS	TBMCS Amides			
		Acylation	MBTFA	Trifluoroacetamides		
			TFAA	Trifluoroacetamides		
PFAA	Pentafluoropropionamides		Good for ECD detection			
HFBA	Heptafluorobutyamides					
Alkylation	MethElute <sup>™</sup> Reagent	Methyl Amides	On-column derivatization especially for drugs			
<b>Amines</b> $\begin{array}{c} \text{H} \\   \\ -\text{C}-\text{NH}_2 \\   \\ \text{H} \\ \text{Primary} \end{array}$	Silylation	BSA	TMS	TMCS aids derivatization		
		BSTFA	TMS			
		BSTFA+TMCS	TMS			
		MSTFA	TMS			
		MSTFA+TMCS	TMS			
	Secondary	Silylation	Tri-Sil <sup>®</sup> Reagents	TMS	TMCS aids derivatization	
			MTBSTFA	TBMCS		
		MTBSTFA+TBMCS	TBMCS			
		Acylation	MBTFA	Trifluoroacetamides		
			TFAA	Trifluoroacetamides		
TFAI	Trifluoroacetamides		Good for trace analysis with ECD			
PFAA	Pentafluoropropionamides	Good for trace analysis with ECD				
PFPI	Pentafluoropropionamides					
HFBI	Heptafluorobutyamides					
Alkylation	MethElute <sup>™</sup>	Methyl Amides	On-column derivatization for specific drugs			
<b>Carbohydrates</b> $(\text{CH}_2\text{OH})_n$	Silylation	MSTFA	TMS	Can be used with some syrups		
		TMSI	TMS			
		Tri-Sil <sup>®</sup> Reagents	TMS			
	Acylation	MBTFA	Trifluoroacetates	Volatile derivatives of mono-, di- and trisaccharides		
TFAI	Trifluoroacetates					
<b>Carboxyl</b> $\begin{array}{c} \text{O} \\    \\ -\text{C}-\text{OH} \end{array}$	Silylation	BSA	TMS	Easily formed, generally not stable, analyze quickly		
		BSTFA	TMS			
		BSTFA+TMCS	TMS			
		MSTFA	TMS			
		TMCS	TMS		Can be used with some salts	
		TMSI	TMS			
		Tri-Sil <sup>®</sup> Reagents	TMS			
		MTBSTFA	TBMCS		More stable than TMS derivatives	
		MTBSTFA+TBMCS	TBMCS		TBMCS aids derivatization	
		Alkylation	PFBBr		Pentafluorobenzyl Esters	Used in EC detection & UV, MS
BF <sub>3</sub> -Methanol	Methyl Esters		Best for large samples of fatty acids			
Methyl-8 <sup>®</sup> Reagent	Methyl Esters		Fatty acids and amino acids			
MethElute <sup>™</sup> Reagent	Methyl Esters		On-column derivatization			
PFAA+Pentafluoropropanol	Pentafluoropropyl Ester	Drug analysis				
<b>Hydroxyl-OH</b> $\text{R}-\text{OH}$ Alcohols	Silylation	BSA	TMS	Most often used derivatives Good thermal stability Poor hydrolytic stability Weak donor usually used with TMCS		
		BSTFA	TMS			
		BSTFA+TMCS	TMS			
		HMDS	TMS			
		MSTFA	TMS			
		MSTFA+TMCS	TMS			
		TMCS	TMS			
		R-OH Alcohols	TMSI		TMS	Weak donor usually used with HMDS; can be used with salts
			Tri-Sil <sup>®</sup> Reagents		TMS	Can be used with syrups
			MTBSTFA		TBMCS	More stable than TMS, good MS fragmentation patterns
 Phenols	Silylation	MTBSTFA+TBMCS	TBMCS	TBMCS aids derivatization		
		Acylation	MBTFA	Trifluoroacetates	Good for trace analysis with EDC	
			TFAA	Trifluoroacetates	Good for trace analysis with EDC	
			TFAI	Trifluoroacetates	Good for trace analysis with EDC	
			PFPI	Pentafluoropropionates	Good for trace analysis with EDC	
	PFAA		Pentafluoropropionates	Good for trace analysis with EDC		
	Alkylation	HFBI	Heptafluorobutrates	Good for trace analysis with EDC		
		HFBA	Heptafluorobutrates	Good for trace analysis with EDC		
		PFBBr	Pentafluorobenzyl Ethers	With alkoxides only		



# Gas Chromatography

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**PIERCE**

# Introduction to Gas Chromatography – the Pierce way.

## Silylation and Silylation Reagents

**No one but Pierce offers the combination of variety, quality and reliability.**

### Derivatization

The chemical literature contains an abundance of data on derivatization, most of which is relevant to particular compounds, classes of compounds and derivatization reagents. Two books are recognized as standards in the field of analytical derivatization. The first book, *Handbook of Analytical Derivatization Reactions* by Daniel R. Knapp<sup>1</sup> (see page 36), provides a general collection of analytical derivatization methods for chromatography and mass spectroscopy (MS) that involves formation of covalent derivatives prior to analysis. The second book, *Silylation of Organic Compounds* by Alan F. Pierce,<sup>2</sup> “is a significant factor in the transfer of silylation reactions from the relatively esoteric field of organosilicon chemistry to the status of perhaps the most widely practiced of derivatization methods.”<sup>3</sup>

Compounds or compound mixtures are derivatized before analysis for the following reasons:

1. To make a compound that otherwise could not be analyzed by a particular method suitable for analysis<sup>4</sup>
2. To improve the analytical efficiency of the compound<sup>5,6</sup>
3. To improve the detectability of the compound<sup>7</sup>

### Suitability

Often compounds cannot be analyzed because they are not in a form that is suitable for the particular analytical technique. Examples include nonvolatile compounds for GC analysis,<sup>8,9,10</sup> insoluble compounds for HPLC analysis, and materials that are not stable using the conditions of the technique.<sup>11</sup> The derivatization procedure modifies the chemical structure of the compounds, allowing analysis by a desired technique.<sup>12</sup>

### Efficiency

Direct analysis can be difficult when compounds interact with each other or with the column. These interactions can lead to poor peak resolution

and/or asymmetrical peaks that make proper peak integration difficult or impractical. This interference can be reduced with conversion to derivatized products.<sup>13,14</sup> Compounds that exhibit co-elution can often be separated by using the appropriate derivatization methods.

### Detectability

As demand increases for the analysis of increasingly smaller amounts of materials, it becomes important to extend the detectability range of the materials in question. This increased sensitivity can be accomplished by improved detector design that is directed toward specific atoms or functional groups.

Another popular approach to increase detectability is the use of derivatization. Enhanced detectability can be achieved by increasing the bulk of the compound, or by introducing atoms or functional groups that strongly interact with the detector.<sup>16,17</sup> This technique is performed in gas chromatographic applications, with the addition of halogen atoms for electron capture detectors,<sup>18,19</sup> and with the formation of TMS derivatives to produce readily identifiable fragmentation patterns and mass ions.<sup>20</sup>

### Types of Derivatization

There are three general types of analytical derivatization reactions used for gas chromatography: silylation, acylation and alkylation. The ideal derivatization procedure will:

1. Accomplish the desired modification.
2. Proceed quantitatively, or at least reproducibly.
3. Produce products that are readily distinguishable and separable from the starting materials.
4. Proceed rapidly with simple and straightforward laboratory techniques, and will be both selective and applicable to a number of similar compounds.
5. Involve reagents and reactions that present no unusual hazards.

Compounds containing functional groups with active hydrogens (-COOH, -OH, -NH and -SH) are usually derivatized for analysis by gas chromatography. These functional groups have a tendency to form intermolecular hydrogen bonds that affect the volatility, their tendency to interact deleteriously with column packing materials and their thermal stability. Silylation, acylation and alkylation are derivatization techniques used to alter these functional groups to improve their thermal and chromatographic character.

Silyl derivatives are the most widely used derivatives for gas chromatographic applications. Usually they are formed by the replacement of the active hydrogens from acids, alcohols, thiols, amines, amides and enolizable ketones and aldehydes with the trimethylsilyl group. A variety of reagents is available for the introduction of the trimethylsilyl group. These reagents differ in their reactivity, selectivity and side reactions and the character of the reaction products from the silylation reagent itself. Considerable literature is available to assist you in the selection of the most suitable silylation reagent for your particular compounds or systems.<sup>1,2</sup>

Silylation reagents and trimethylsilyl derivatives are hydrolytically unstable and must be protected from moisture. However, the rate of hydrolysis for various reagents and derivatives is different, and sometimes it is possible to prepare derivatives in the presence of small amounts of moisture,<sup>21</sup> or to isolate and purify derivatives by extraction in an organic solvent, followed by washing with aqueous solutions.<sup>22</sup> Reagents that introduce a *t*-butyldimethylsilyl group instead of the trimethylsilyl group were developed for greater hydrolytic stability.<sup>23</sup> These derivatives provide improved stability against hydrolysis and provide distinctive fragmentation patterns, making them useful in GC/MS applications.<sup>24</sup>

Most trimethylsilyl and *t*-butyldimethylsilyl derivatives offer excellent thermal stability and are suitable for a wide range of injector and column conditions. However, as the silylation reagents will derivatize nearly all active hydrogens, it is

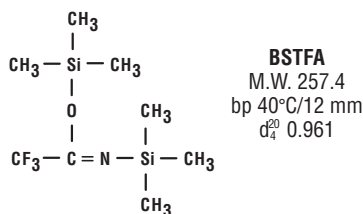
important that they are not injected onto any column in which the stationary phase contains these functional groups. Examples of packings that are not compatible with silylating reagents are polyethylene glycols (Carbowax<sup>®</sup> Glycols and Superwax) and free fatty acid phases (FFAP).

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## BSTFA

For excellent chromatographic separations.



The greatest advantage of using BSTFA over other silylating reagents is the increased volatility of its byproducts, mono(trimethylsilyl) trifluoroacetamide and trifluoroacetamide. This increased volatility results in the byproducts eluting with the solvent front, providing excellent chromatographic separations.

BSTFA is a powerful trimethylsilyl donor, with donor strength that is comparable to its unfluorinated analog BSA [N,O-bis(trimethylsilyl)acetamide]. BSTFA reacts to replace labile hydrogens on a wide range of polar compounds with a  $-\text{Si}(\text{CH}_3)_3$  group. This physical characteristic is particularly useful in the gas chromatography of some lower boiling TMS-amino acids and TMS Krebs cycle acids.

### PROTOCOL

1. Combine 5-10 mg sample, 0.5 ml BSTFA and 1.0 ml solvent (acetonitrile is recommended for amino acids) in a 3.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Cap vial and shake for 30 seconds.
3. Heat at 70°C for 15 minutes.
4. Analyze by gas chromatography.

**NOTE:** This protocol is not recommended for sugars.

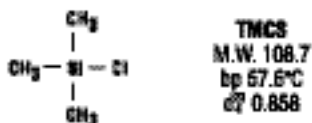
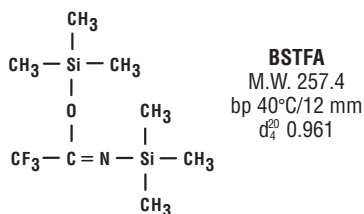
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### Ordering Information

Product #	Description	Pkg. Size
38828	<b>BSTFA</b> [N, O-bis(trimethylsilyl)trifluoroacetamide]	25 g Hypo-Vial™ Sample Storage Vial
38829	<b>BSTFA</b>	100 g Hypo-Vial™ Sample Storage Vial
38830	<b>BSTFA</b>	10 x 1 ml ampules

## The reagent to choose for difficult-to-silylate compounds.



BSTFA + 1% TMCS is ideal for derivatizing fatty acid amides, slightly hindered hydroxyls and other difficult-to-silylate compounds. This catalyzed formulation is stronger than BSTFA alone.

### PROTOCOL

- Combine 5-10 mg sample, 0.5 ml BSTFA + 1% TMCS and 1.0 ml solvent (acetonitrile is recommended for amino acids) in a 3.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
- Cap vial and shake for 30 seconds.
- Heat at 7°C for 15 minutes.
- Analyze by gas chromatography.

### References

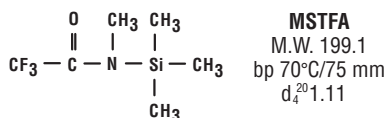
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### Ordering Information

Product #	Description	Pkg. Size
38831	<b>BSTFA + 1% TMCS</b> [N-O-bis(Trimethylsilyl)trifluoroacetamide + 1% Trimethylchlorosilane]	10 x 1 ml ampules
38832	<b>BSTFA + 1% TMCS</b>	10 g Hypo-Vial™ Sample Storage Vial
38833	<b>BSTFA + 1% TMCS</b>	25 g Hypo-Vial™ Sample Storage Vial
38834	<b>BSTFA + 1% TMCS</b>	100 g Hypo-Vial™ Sample Storage Vial
38840	<b>BSTFA + 10% TMCS</b> [N-O-bis(Trimethylsilyl)trifluoroacetamide + 10% Trimethylchlorosilane]	10 x 1 ml ampules

# MSTFA<sup>1-9</sup> and MSTFA 1% TMCS<sup>10-12</sup>

**Offers maximum volatility.**



MSTFA is the most volatile TMS-amide available – its even more volatile than BSTFA or BSA.<sup>1</sup> Its byproduct, *N*-methyltrifluoroacetamide, has a lower retention time in GC applications than MSTFA itself. This makes it ideal for GC determinations in which the reagent or byproducts may obscure the derivative on the chromatogram. Silylation of steroids shows MSTFA to be significantly stronger in donor strength than BSTFA or BSA.<sup>2</sup> MSTFA will silylate hydrochloride salts of amines directly.

## PROTOCOL

1. Combine 5-10 mg sample, 0.5 ml MSTFA and 1.0 ml solvent (acetonitrile is recommended for amino acids) in a 3.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Cap vial and shake for 30 seconds.
3. Heat at 70°C for 15 minutes.
4. Analyze by gas chromatography.

## References

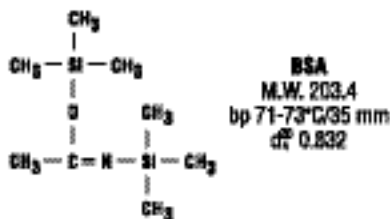
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## Ordering Information

Product #	Description	Pkg. Size
✘ 48910	MSTFA ( <i>N</i> -Methyl- <i>N</i> -trimethylsilyltrifluoroacetamide)	10 x 1 ml ampules
✘ 48911	MSTFA	10 g Hypo-Vial™ Sample Storage Vial
✘ 48915	MSTFA + 1% TMCS ( <i>N</i> -Methyl- <i>N</i> -trimethylsilyltrifluoroacetamide + 1% Trimethylchlorosilane)	10 x 1 ml ampules

✘ Additional hazardous handling charge.

## The perfect reagent for volatile TMS derivatives.



Under relatively mild conditions, BSA reacts quantitatively with a wide variety of compounds to form volatile, stable TMS derivatives for GC analysis. BSA is used extensively for derivatizing alcohols, amines, carboxylic acids, phenols, steroids, biogenic amines and alkaloids. It is not recommended for use with carbohydrates or very low molecular weight compounds.

BSA is used in conjunction with a solvent such as pyridine or DMF, and reactions are generally rapid. When used with DMF, BSA is the most suitable reagent for derivatizing phenols. A study of the silylating properties of BSA made by Klebe, Finkbeiner and White<sup>1</sup> showed the following reactions with BSA:

- Amino acids to form both N,O bonded TMS derivatives
- Hydroxyl compounds to form TMS ethers
- Organic acids to form TMS esters
- Aromatic amides to form N-TMS derivatives

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## Ordering Information

Product #	Description	Pkg. Size
38836	BSA [N,O-bis(Trimethylsilyl)acetamide]	10 x 1 ml ampules
38838	BSA	25 g Hypo-Vial™ Sample Storage Vial
38839	BSA	100 g Hypo-Vial™ Sample Storage Vial

### PROTOCOL 1

1. Combine 5-10 mg sample, 0.5 ml BSA and 1.0 ml solvent (acetonitrile is recommended for amino acids) in a 3.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Cap vial and shake for 30 seconds.
3. Heat at 70°C for 15 minutes.
4. Analyze by gas chromatography.

### PROTOCOL 2

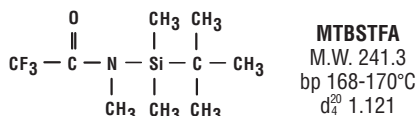
This method was developed by E.M. Chambaz and E.C. Horning for the silylation of hydroxyl groups in sterically unhindered positions in steroids. This includes sites such as 3, 7, 16, 17(sec), 20 and 21 positions in the steroid structure. This method may be used for silylating many hydroxyl and polyhydroxyl compounds other than steroids. It is not recommended, however, for sugars. The method is based upon the use of BSA in an uncatalyzed reaction. No trimethylchlorosilane should be used in this reaction. Hydrochlorides should be avoided because HCl also will act as a catalyst.

1. Combine 0.1-5.0 mg of sample and 0.2-0.4 ml BSA in a 1.0 ml Reacti-Vial™ Small Reaction Vial (see page 68). If material is not soluble in BSA, add 0.1-0.2 ml pyridine.
2. Cap vial and shake for 30 seconds.
3. Heat at 60°C to ease dissolution, if desired.

**NOTE:** Material is silylated at room temperature within times varying from a few minutes to a few hours. Heating will hasten reaction.

## MTBSTFA

**Offers stable TBDMS (*tert*-butyldimethylsilyl) derivatization.**



- Derivatizes hydroxyl, carboxyl, thiol and primary and secondary amines
- Rapid reaction times (5-20 minutes)
- Typical yield >96%; TBDMS ethers are 10<sup>4</sup> times more stable than TMS ethers<sup>1</sup>
- Neutral, volatile byproducts
- High molecular ion concentration at M-57

*N*-Methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) provides TBDMS derivatives without the disadvantage of earlier reported TBDMS-Cl formulations. Bazan and Knapp have demonstrated the usefulness of MTBSTFA by preparing an improved derivative of 6-keto-prostaglandin F1 for GC-MS.<sup>2</sup>

### PROTOCOL

1. Combine 1-10 mg of sample, 0.1 ml MTBSTFA and 0.1 ml acetonitrile in a 1.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Cap vial and stand at room temperature 5-20 minutes.
3. Analyze by gas chromatography.

**NOTE:** Other solvents may be used including DMF, pyridine and THF. DMF is not recommended for primary or secondary amines.)

### References

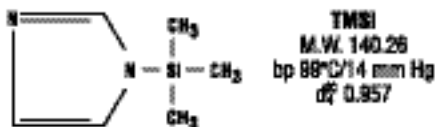
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### Ordering Information

Product #	Description	Pkg. Size
✗ 48920	MTBSTFA [ <i>N</i> -methyl- <i>N</i> -( <i>tert</i> -butyldimethylsilyl)trifluoroacetamide]	5 ml specially purified

✗ Additional hazardous handling charge.

## The strongest hydroxyl silylator available for carbohydrates and steroids.



Sakauchi and Horning have shown TMSI to be an all-purpose reagent for unhindered steroids to highly hindered steroids.<sup>2</sup>

TMSI is unique, as it reacts quickly and smoothly with hydroxyls and carboxylic acids, but not with amines.<sup>3,4</sup> Because TMS-derivatives are less stable than TMS-ethers or -esters, TMSI is especially useful in multidervatization schemes for compounds containing both hydroxyl and amine groups (such as in the preparation of -O-TMS, -N-HFB derivatives of catecholamines).<sup>4</sup>

TMSI is used in the derivatization of alcohols, phenols, organic acids steroids hormones glycols, nucleotides and narcotics. In addition, it is excellent for C1 through C5 fatty acids in serum and urine.<sup>5</sup>

### References

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2. Sakauchi, N. and Horning, E.G. (1971). *Anal. Lett.* **4**(1), 41-52.
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16. Yoo, Y., *et al.* (1995). Determination of nalbuphine in drug abusers' urine. *J. Anal. Toxicol.* **19**, 120.
17. Seidel, V., *et al.* (1993). Analysis of trace levels of trichothecene mycotoxins in Austrian cereals by gas chromatography with electron capture detection. *Chromatographia* **37**, 191.

### PROTOCOL 1

This method combines silylation of hydroxyl groups and acylation of amino groups. It was first used by M.G. Horning, *et al.* to prepare catecholamines for GC and GC/MS determinations.<sup>4</sup> This method takes advantage of the fact that TMSI will silylate only hydroxyl groups. Effectively, this blocks those sites from acylation while leaving the amine sites open for acylation.

1. Combine and dissolve 1.0 mg sample and 1.0 ml acetonitrile in a 1.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Add 0.2 ml TMSI.
3. Cap vial and heat at 60°C for 3 hours.
4. Add 0.1 ml HFBI, TEAL or PFPI (depending on which acyl derivative is desired).
5. Cap vial and heat at 60°C for 30 minutes.
6. Analyze by gas chromatography.

### PROTOCOL 2

This method was developed by Sakauchi and Horning for the silylation of hydroxyl groups on highly hindered steroids. It offers fast conversion to TMS-ethers at a moderate temperature with a single reagent.

1. Combine 0.1-5.0 mg of steroid, 0.1-1.0 ml TMSI (0.1 ml pyridine should be added for solubilization of cortol and cortolones) in a 1.0 or 3.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Cap vial and heat at 100°C for 2 hours.
3. Analyze by gas chromatography.

### PROTOCOL 3

1. Combine 400 µl TMSI and 800 µl pyridine (other solvents may be used) in a 3.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Add 10-15 mg sample.
3. Cap vial and shake until sample is dissolved. Heat to 60-70°C if needed.
4. Analyze by gas chromatography.

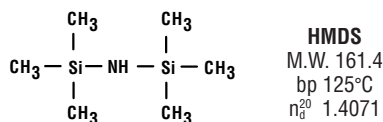
**NOTE:** TMSI may be used straight with carbohydrates or as a 50% solution with pyridine for wet sugars.

## Ordering Information

Product #	Description	Pkg. Size
✗ 88623	<b>TMSI</b> (N-Trimethylsilylimidazole)	10 x 1 ml ampules
88625	<b>TMSI</b>	25 g Hypo-Vial™ Sample Storage Vial
✗ 88626	<b>TMSI</b>	100 g Hypo-Vial™ Sample Storage Vial

✗ Additional hazardous handling charge.

## HMDS

The popular choice for silylation of sugars and related substances.<sup>1</sup>

HMDS greatly extends the practical range of GC, improving chromatographic results in the silylation of sugars and related substances.

A critical study of the optimal proportions of HMDS and trimethylchlorosilane for producing maximum yield of trimethylsilyl derivatives was conducted by Sweeley, *et al.*<sup>1</sup>

### PROTOCOL 1

This protocol describes the method of Sweeley, *et al.* for the trimethylsilylation of sugars and related substances.<sup>1</sup>

1. Combine 10 mg or less carbohydrate sample, 1.0 ml anhydrous pyridine, 0.2 ml HMDS and 0.1 ml TMCS in a 3.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Cap vial and shake vigorously 30 seconds.
3. Let stand at room temperature 5 minutes or until derivatization is complete.
4. Analyze by gas chromatography.

**NOTE:** Solution may become cloudy when TMCS is added, due to fine precipitate of ammonium chloride. Precipitate will not interfere with gas chromatography. Carbohydrates may be warmed for 10-20 minutes at 75-85°C to hasten dissolution.

### PROTOCOL 2

This method was developed primarily for silylating syrups and concentrated aqueous solutions of sugars such as starch hydrolyzates.<sup>2</sup>

**CAUTION:** Considerable heat, ammonia gas and pressure emit during reaction. Do not premix.

1. Place 60-70 mg of 80% solids syrup in 3.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Add 1.0 ml pyridine and dissolve.
3. Add 0.9 ml HMDS and mix.
4. Add 0.1 ml trifluoroacetic acid.
5. Shake vigorously 30 seconds.
6. Let stand 15 minutes.
7. Analyze by gas chromatography.

### References

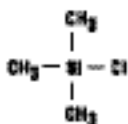
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2. Brobst, K.M. and Lott, C.E., Jr. (1966). *Cereal Chem.* **43**, 35-43.
3. Gehrke, C.W., *et al.* (1970). Trimethylsilylation of amino acids. Effects of solvents on derivatization using Bis(trimethylsilyl)-Trifluoroacetamide. *J. Chromatogr.* **53**, 201-208.
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14. Ogden, M.W., *et al.* (1986). Characterization of fused-silica capillary tubing by contact angle measurements. *J. Chromatogr.* **354**, 7-18.
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## Ordering Information

Product #	Description	Pkg. Size
✗ 84770	HMDS (Hexamethyldisilazane)	25 g Hypo-Vial™ Sample Storage Vial
✗ 84769	HMDS	100 g Hypo-Vial™ Sample Storage Vial

✗ Additional hazardous handling charge.

**An excellent catalyst for difficult-to-silylate compounds.**



**TMCS**  
 M.W. 108.7  
 bp 57.6°C  
 d<sub>4</sub><sup>20</sup> 0.858

TMCS (trimethylchlorosilane) provides an excellent adjunct for forming trimethylsilyl ethers for GC determinations.<sup>1,2,3</sup> In addition, it is used for preparing TMS derivatives of organic acids.<sup>4,5</sup>

## PROTOCOL

This protocol describes the method of Sweeley, *et al.* for the trimethylsilylation of sugars and related substances.<sup>1</sup>

1. Combine 10 mg or less carbohydrate sample, 1.0 ml anhydrous pyridine, 0.2 ml HMDS and 0.1 ml TMCS in a 3.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Cap vial and shake vigorously 30 seconds.
3. Let stand at room temperature 5 minutes or until derivatization is complete.
4. Analyze by gas chromatography.

**NOTE:** Solution may become cloudy when TMCS is added due to fine precipitate of ammonium chloride. Precipitate will not interfere with gas chromatography. Carbohydrates may be warmed for 10-20 minutes at 75-85°C to hasten dissolution.

## References

1. Sweeley, C.C., *et al.* (1963). *JACS* **85**, 2497-2507.
2. Hedgley, E.V. and Overend, W.G. (1960). *Chem. and Ind. (London)*. **378**.
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## Ordering Information

Product #	Description	Pkg. Size
✖ 88530	TMCS	25 g Hypo-Vial™ Sample Storage Vial

✖ Additional hazardous handling charge.

# MOX™ Reagent

**Use this reagent for preparing oximes of steroids and ketoacids prior to silylation.**

MOX™ Reagent (M.W. 83.51) converts keto groups to methoxime derivatives. It prevents the formation of multiple derivatives (which interfere with quantitation) when enols are present during silylation. MOX™ Reagent is a 2% solution of methoxyamine•HCl in pyridine, and it is used primarily with steroids.

The procedures below are used to prepare methoxime derivatives of steroids and ketoacids prior to silylation. Forming methoximes is based on the work of Fates and Luukkainen, with further applications by Horning, *et al.* Both procedures have been used successfully by Horning, *et al.*<sup>1</sup>

## PROTOCOL 1

This simplified procedure is for stable ketones that are readily soluble in organic solvent.

1. Combine 2 mg sample and 0.5 ml MOX™ Reagent in a 10 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Cap vial and heat at 60°C for 3 hours.
3. Add 2 ml water.
4. Extract with three 5 ml portions of high-purity benzene.
5. Combine benzene extracts and wash with 1 N HCl, followed by bicarbonate solution.
6. Dry over anhydrous magnesium sulfate and evaporate to 0.5 ml with nitrogen.
7. Analyze by gas or thin layer chromatography.

## PROTOCOL 2

This procedure is for polar steroids, such as corticoids, that have several hydroxyl groups.<sup>2</sup>

1. Combine 2 mg sample and 0.5 ml MOX™ Reagent in a 10 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Cap vial and let stand overnight at room temperature.
3. Add 2 ml saturated NaCl solution.
4. Extract with three 5 ml portions of high-purity ethyl acetate.
5. Combine ethyl acetate extracts and wash with salt saturated 0.1 N HCl wash, followed by a 5% NaHCO<sub>3</sub> salt saturated wash.
6. Dry over anhydrous magnesium sulfate and evaporate with nitrogen to 0.5 ml.
7. Analyze by gas or thin layer chromatography.

**NOTE:** After completing the methoxime reaction, some researchers have silylated the reacted mixture without further treatment. The resulting mixture was centrifuged to remove solids, and aliquots of the sample were used for gas chromatography.

## References

1. Horning, M.G., *et al.* (1968). *Anal. Biochem.* **22**, 284.
2. Maume, B., *et al.* (1968). *Anal. Lett.* **1**, 401.
3. Laine, R.A., *et al.* (1971). Analysis of trimethylsilyl-O-methyloximes of carbohydrates by combined gas-liquid chromatography-mass spectrometry. *Anal. Biochem.* **43**, 533-538.
4. Benko, A.B., *et al.* (1980). Comparison of silylation reaction rates of different reagents: catalytic effect of methoxyamine on the silylation of sterically hindered hydroxyl groups. *Anal. Lett.* **13(A9)**, 735-739.
5. Sabot, J.F., *et al.* (1985). Determination of plasma testosterone by mass fragmentography using [3,4-<sup>13</sup>C] testosterone as an internal standard. *J. Chromatogr.* **339**, 233-242.
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## Ordering Information

Product #	Description	Pkg. Size
✗ 45950	MOX™ Reagent	10 ml Hypo-Vial™ Sample Storage Vial

✗ Additional hazardous handling charge.

## For preparing oximes of sugars prior to silylation.

STOX™ Reagent (M.W. 83.51) is used primarily with sugars. It is a pyridine solution containing 25 mg/ml hydroxylamine hydrochloride and 6 mg/ml phenyl-β-D-glucopyranoside as an internal standard.

### PROTOCOL

This method is designed to assist in the routine determination of sugars in food products and syrup. Sugars are treated with hydroxylamine hydrochloride. The resulting oximes are converted to TMS ethers. Oximes are silylated directly – the results are quantitative and reproducible. Multiple peaks, due to the tautomeric forms of reducing sugar, are eliminated. Syrups containing 20-30% (or more) water need not be concentrated prior to analysis as long as no more than 20 mg of water are present in the sample to be derivatized. Samples containing 70-75% water should be concentrated.

1. Combine 10-15 mg sugar mixture and 1.0 ml STOX™ Reagent in a 3.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Cap vial and heat at 70-75°C for 30 minutes. Proceed to 3a or 3b.
- 3a. Cool to room temperature. Add 1.0 ml HMDS and mix. Add 0.1 ml TFA. Cap vial and shake for 30 seconds. React at room temperature for 30 minutes to allow the white precipitate to settle.
- 3b. Cool to room temperature. Add 1.0 ml TMSI. Cap vial and shake for 30 seconds. React for 30 minutes.
4. Analyze by GC.

### References

1. Mason, B.S., *et al.* (1971). A gas chromatographic method for the determination of sugars in foods. *J. Agr. Food Chem.* **19**(3).
2. Anderle, D., *et al.* (1977). Separation of trioses and tetroses as trimethylsilyl oximes by gas chromatography. *Anal. Chem.* **49**(1).
3. Marcy, J.E., *et al.* (1982). A rapid method for the simultaneous determination of major organic acids and sugars in grape musts. *Am. J. Enol. Viticult.* **33**(3).

## Ordering Information

Product #	Description	Pkg. Size
49805	STOX™ Reagent Oxime-Internal Standard Reagent	50 ml Hypo-Vial™ Sample Storage Vial

# The Tri-Sil® Family of Reagents – an easy choice for purity and convenience.

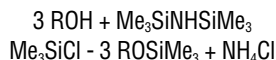
## Tri-Sil® Reagent

### Our reagent-catalyst solvent mixture for one-step derivatization.

Tri-Sil® Reagent is composed of HMDS, TMCS and high purity pyridine. It is useful for rapid production of TMS derivatives of polar compounds for gas chromatographic determination and biochemical synthesis. Tri-Sil® Reagent is ideal for GC determinations of:

- Sugars<sup>1-6</sup>
- Alcohols<sup>1</sup>
- Phenols<sup>7</sup>
- Steroids<sup>8,9</sup>
- Sterols<sup>10,11</sup>
- Bile acids and other organic acids<sup>12-14</sup>
- Some amines<sup>15-17</sup>

Tri-Sil® Reagent is based on the procedure of Sweeley, *et al.*<sup>1</sup> and is used for the optimal conversion of organic hydroxyl and polyhydroxyl compounds into TMS ethers. The reaction proceeds as:



The versatility, speed and ease of use of our Tri-Sil® Reagent has made it the most widely used silylation formulation available.

### PROTOCOL

1. Combine 5-10 mg sample and 1.0 ml Tri-Sil® Reagent in a 3.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Shake the reaction vigorously for 30 seconds or warm to 75-85°C to dissolve.
3. React at room temperature for 5 minutes.
4. Analyze by gas chromatography.

**NOTE:** A majority of hydroxyl and polyhydroxyl compounds will be completely derivatized in less than 5 minutes including sugars, phenols, organic acids, some amines and alcohols. Highly hindered compounds, such as some steroids, may require 15 minutes to 8 hours. Extremely intractable compounds may require refluxing for several hours.

### References

1. Sweeley, C.C., *et al.* (1963). *JACS* **85**, 2497-2507.
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12. Makita, M. and Wells, W.W. (1963). *Anal. Biochem.* **5**, 523.
13. Burkhard, C.A. (1957). *J. Org. Chem.* **22**, 592.
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19. Ng, L., *et al.* (1993). Simple gas chromatographic method for the assay of salts of carboxylic acids as their trimethylsilyl derivatives. *J. Chromatogr.* **637**, 104.

### Ordering Information

Product #	Description	Pkg. Size
✘ 48999	Tri-Sil® Reagent	10 x 1 ml ampules
✘ 49001	Tri-Sil® Reagent	50 ml Hypo-Vial™ Sample Storage Vial

✘ Additional hazardous handling charge.

## A reagent-solvent solution for one-step derivatization.

Tri-Sil<sup>®</sup> BSA Formulation P is composed of BSA (2.5 mEq/ml\*) and Pyridine.

\*1.25 mEq for amides

Tri-Sil<sup>®</sup> BSA reacts with:

- Alcohols phenols, some enols and other hydroxyl and polyhydroxyl compounds to form trimethylsilyl ethers
- Organic acids to form trimethylsilyl esters
- Aromatic amides to form *N*-trimethylsilyl derivatives
- Amines to form *N*-trimethylsilyl derivatives

In addition, Tri-Sil<sup>®</sup> BSA is excellent for unhindered steroids, but it is not recommended for carbohydrates.

### PROTOCOL

1. Combine 5-10 mg sample and 1.0 ml Tri-Sil<sup>®</sup> BSA in a 3.0 ml Reacti-Vial<sup>™</sup> Small Reaction Vial (see page 68).
2. Cap vial and heat at 60-70°C for 15-20 minutes to facilitate dissolution and derivatization.
3. Analyze by gas chromatography.

### References

1. Fennessey, P.V., *et al.* (1980). Rearrangements of the TMS derivatives of acylglycines. *Org. Mass Spec.* **15**(4).
2. Ramsdell, H.S., *et al.* (1980). Gas chromatographic retention indices of twenty metabolically important acylglycines as trimethylsilyl derivatives. *J. Chromatogr.* **181**, 90-94.

### Ordering Information

Product #	Description	Pkg. Size
✗ 49012	Tri-Sil <sup>®</sup> BSA, Formula "P" (in Pyridine)	25 ml Hypo-Vial <sup>™</sup> Sample Storage Vial

✗ Additional hazardous handling charge.

# Tri-Sil<sup>®</sup> TBT

## A powerful catalyzed silylation reagent formulation.

Tri-Sil<sup>®</sup> TBT is a mixture containing three parts TMSI, three parts BSA and two parts TMCS. Tri-Sil<sup>®</sup> TBT converts all classes of hydroxyl groups to TMS ethers. Under usual conditions, the reaction is complete in a short period of time at 60-80°C. Highly hindered hydroxyls may require several hours.

### References

1. Huizing, H.J., *et al.* (1986). Positive and negative ion chemical ionization mass spectrometry of trimethylsilyl derivatives of pyrrolizidine alkaloids using NH<sup>+</sup> or OH<sup>-</sup> as the reactant ions. *Biomed. and Environ. Mass Spec.* **13**, 293-298.
2. Seidel, V., *et al.* (1993). Analysis of trace levels of trichothecene mycotoxins in Austrian cereals by gas chromatography with election capture detection. *Chromatographia* **37**, 191.

### PROTOCOL

This method is used to silylate all hydroxyl groups in steroid structures, even the most sterically hindered, such as the 17 hydroxyl groups in cortol. This method also has been used by Bacon and Kokenakes to measure plasma prednisolone by GC.

1. Combine 0.1-5.0 mg sample and 0.2-0.4 ml Tri-Sil<sup>®</sup> TBT in a 1.0 ml Reacti-Vial<sup>™</sup> Small Reaction Vial (see page 68).
2. Cap vial and shake to dissolve.
3. Heat at 60-80°C for 6-24 hours to complete reaction.
4. Analyze by gas chromatography.

**NOTE:** If sample is insoluble, add 0.1-0.2 ml pyridine.

### Ordering Information

Product #	Description	Pkg. Size
✗ 49016	Tri-Sil <sup>®</sup> TBT (in Pyridine)	10 x 1 ml ampule

✗ Additional hazardous handling charge.

# Tri-Sil<sup>®</sup> Z

## Great for derivatizing hydroxyl compounds.

Tri-Sil<sup>®</sup> Z is a mixture of TMSI in dry pyridine (1.5 mEq/ml). It is used primarily for derivatizing hydroxyl compounds, particularly carbohydrates. Tri-Sil<sup>®</sup> Z has been used successfully for the silylation of alcohols and phenols, organic acids, hydroxylamines, amino acids, carbohydrates, flavonoids, glycols and polyglycols, nucleotides, steroids, hydroxyl acids, barbiturates, narcotics, indoles, and vitamins. Tri-Sil<sup>®</sup> Z does not react with amines.

Tri-Sil<sup>®</sup> Z can be used in the presence of water as long as there is enough reagent present to react with both the water and the sample. The reagent reads with water in a 2:1 ratio.

### PROTOCOL 1

1. Combine 10-15 mg sample and 1.0 ml Tri-Sil<sup>®</sup> Z in a 3.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Cap vial and shake to dissolve. If necessary, heat at 60-70°C. Silylation is complete upon dissolution.
3. Analyze by gas chromatography.

### PROTOCOL 2

For solutions containing ~1% or less total sugars, use a 50:50 v/v TMSI/pyridine solution.

1. Evaporate ~50 µl sugar solution to a glassy syrup in a 0.3 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Add 50 µl TMSI/pyridine solution.
3. Cap vial and heat at 60°C to dissolve and derivatize the sugars.
4. Analyze directly by gas chromatography.

### References

1. Hymowitz, T., *et al.* (1972). Relationship between the content of oil, protein and sugar in soybean seed. *Agronomy J.* **64**, 613-616.
2. Mamer, O. and Gibbs, B. (1973). *Clin. Chem.* **19(9)**, 1006-1009.
3. Quilliam, M.A., *et al.* (1980). Study of rearrangement reactions occurring during gas chromatography of *tert*-butyldimethylsilyl ether derivatives of uridine. *J. Chromatogr.* **194**, 379-386.
4. Low, N., *et al.* (1994). Normative data for commercial pineapple juice from concentrate. *J. of AOAC International* **77**, 965.

## Ordering Information

Product #	Description	Pkg. Size
✘ 49230	Tri-Sil <sup>®</sup> Z	10 x 1 ml ampules
✘ 49231	Tri-Sil <sup>®</sup> Z	25 ml Hypo-Vial™ Sample Storage Vial

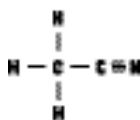
✘ Additional hazardous handling charge.

# Silylation Grade Solvents

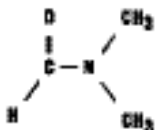
**Manufactured to meet your exacting silylation needs.**

Pierce Silylation Grade Solvents are specially manufactured and packaged to meet the exacting needs of silylation and other sensitive derivatization reactions. Each Silylation Grade Solvent is purified, dried and packaged under nitrogen

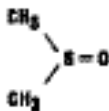
in our convenient Hypo-Vial™ Sample Storage Vials (see page 70). Supplied complete with elastomer septa, this unique packaging allows immediate access to your sample, without exposure to moisture and oxygen.



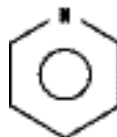
**Acetonitrile**  
M.W. 41.05  
bp 81.6°C



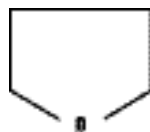
**Dimethylformamide**  
M.W. 73.09  
bp 153°C



**Dimethylsulfoxide**  
M.W. 78.13  
bp 189°C



**Pyridine**  
M.W. 79.10  
bp 115.2°C



**Tetrahydrofuran**  
M.W. 72.10  
bp 66°C

- Purified, dried and packaged under nitrogen in convenient Hypo-Vial™ Sample Storage Vials
- Supplied with elastomer septa, allowing immediate access to sample without exposure to moisture and oxygen
- Use polar solvents (acetonitrile, dimethylformamide, dimethylsulfoxide, pyridine and tetrahydrofuran) to facilitate reactions; nonpolar organic solvent may be used, but they will not accelerate the rate of reaction
- Avoid water or alcohol because TMS reagents react with active hydrogen; avoid enolizable ketones
- Use dimethylformamide for steroids and other large molecules
- Use dimethylsulfoxide to prepare TMS derivatives of tertiary alcohols and some compound with reluctant solubility in other silylation solvents
- Pyridine is an excellent solvent and reaction medium for MS reactions and is an HCl acceptor in reactions involving organochlorosilanes
- Other commonly used solvents include tetrahydrofuran and acetonitrile

## Ordering Information

Product #	Description	Pkg. Size
✗ 20062	<b>Acetonitrile</b>	50 ml Hypo-Vial™ Sample Storage Vial
✗ 20672	<b>Dimethylformamide</b>	50 ml Hypo-Vial™ Sample Storage Vial
20684	<b>Dimethylsulfoxide</b>	50 ml Hypo-Vial™ Sample Storage Vial
✗ 27530	<b>Pyridine</b>	50 ml Hypo-Vial™ Sample Storage Vial
✗ 27860	<b>Tetrahydrofuran</b>	50 ml Hypo-Vial™ Sample Storage Vial

✗ Additional hazardous handling charge.

# Introduction to Pierce Acylation Reagents

Acylation is the conversion of compounds (through the action of a carboxylic acid or a carboxylic acid derivative) that contain active hydrogens such as -OH, -SH and -NH esters; thioesters; and amides.<sup>1</sup> In chromatographic applications, the acylation reaction is used primarily for converting the above classes of compounds into derivatives that are better suited for chromatography<sup>2</sup> or that give a greater response to the chromatographic detection system than the parent compound.<sup>3</sup>

An important example of this application is the insertion of perfluoroacyl groups into a molecule to enhance the detectability of the substance by electron capture. The presence of a carbonyl group adjacent to the halogenated carbons enhances the electron capture detector (ECD) response.

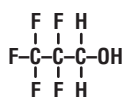
Acyl derivatives also are useful in MS applications in which they direct the fragmentation patterns of the compounds to be studied.<sup>4</sup>

## References

1. Donike, M. (1973). Acylation with *bis*(acylamides). *N*-Methyl-*bis*(Trifluoroacetamide), two new reagents for trifluoroacetylation. *J. Chromatogr.* **78**, 273-279.
2. Sullivan, J.E. and Schewe, L.R. (1977). Preparation and gas chromatography of highly volatile trifluoroacetylated carbohydrates using *N*-Methyl-*bis*(Trifluoroacetamide). *J. Chromatogr. Sci.* **15**, 196-197.
3. Benington, F., *et al.* (1975). Identification and separation of indolealkylamines by gas liquid chromatographic analysis of their heptafluorobutyl derivatives. *J. Chromatogr.* **106**, 435-439.
4. Borga, O., *et al.* (1971). Quantitative determination of nortriptyline and desmethylnortriptyline in human plasma by combined gas chromatography-mass spectrometry. *J. Chromatogr.* **4**(12), 837-849.

## Pentafluoropropanol

Purified for GC/MS use.



### Pentafluoropropanol

M.W. 150.05  
bp 80.6°C  
 $d_4^{20}$  1.2880

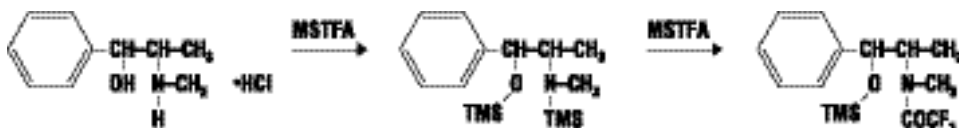
- Addition of fluorine atoms into compounds greatly enhances the sensitivity of certain detectors for those materials
- It is advantageous to introduce fluorine atoms for ECD and GC/MS applications
- Carboxylic acids can be derivatized using a two-step reaction involving reaction with an anhydride, followed by a fluorinated alcohol

## Ordering Information

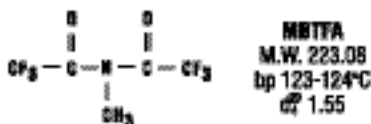
Product #	Description	Pkg. Size
65195	Pentafluoropropanol	10 x 1 ml ampules

# MBTFA

**For trifluoroacetylating primary and secondary amines, hydroxyl and thiol groups, and carbohydrates.**



Selective acylation of amine groups in the presence of hydroxyl and carboxyl groups is possible if these groups are first protected by silylation. The multifunctional compound first is silylated with MSTFA (*N*-Methyl-*N*[(TMS) trifluoroacetamide], which silylates all of the functional groups. The compound then is further reacted with MBTFA, exchanging the TMS-group on the amino function with a trifluoroacetyl group. Similar results are obtained with amino acids that yield *N*-Trifluoroacetyl-O-TMS-esters.



MBTFA is ideal for trifluoroacetylating primary and secondary amines, hydroxyl and thiol groups, or carbohydrates under nonacidic conditions.<sup>1</sup> In addition, MBTFA is used to selectively acylate amines in the presence of hydroxyl and carboxyl functions. The reaction byproduct, *N*-Methyltrifluoroacetamide, is volatile. MBTFA also produces very volatile derivatives of carbohydrates.<sup>2</sup>

## PROTOCOL 1

For trifluoroacetylating primary and secondary amines, and hydroxyl and thiol groups.

- Combine 1-10 mg sample and 0.1-0.2 ml MBTFA. If sample is not easily solubilized, add 0.5-1.0 ml pyridine, DMF, THF or acetonitrile. (MBTFA can be pre-mixed with solvent in a 1:4 ratio. Add 1 ml pre-mixed solution to 1-10 mg compound.)
- Cap vial and heat at 60-100°C for 10-30 minutes (longer for hindered compounds).
- Cool and analyze by gas chromatography.

**NOTE:** MBTFA reacts with amines at room temperature to yield quantitative results in approximately 30 minutes. Hydroxyl groups are slower to react. As a general procedure, reaction mixtures should be heated 10-30 minutes at 60-100°C. In the case of hindered compounds, further heating may be necessary.

## PROTOCOL 2

For trifluoroacetylating sugars.<sup>2</sup>

Producing TFA derivatives of sugars using standard fluorinated anhydride and fluorinated acylimidazole procedures has yielded multiple or unstable derivatives. MBTFA produces the corresponding trifluoroacetyl derivatives of the mono-, di-, tri- and tetrasaccharides. These derivatives, when subjected to gas chromatography, produce quantitative results and yielded an unexpectedly high degree of volatility.

The high volatility of the corresponding TFA derivatives yields shorter retention times at lower temperatures than other commonly used silylation methods. The result is that polar columns with lower maximum temperature limits can be used to provide faster separations under less stringent chromatographic conditions. Mixtures of carbohydrates containing mono- through tetrasaccharides can be analyzed in a single run in as little as 15 minutes.

- Combine 5-10 mg dry sugar and 0.5 ml MBTFA in a 5 ml Reacti-Vial™ Small Reaction Vial.
- Add 0.5 ml pyridine.
- Cap vial and heat at 65°C for 1 hour with occasional shaking.
- Analyze 1 µl by gas chromatography.

**NOTE:** Reactions are complete upon dissolution.

## References

- Donike, M. (1973). *N*-Methyl-bis(Trifluoroacetamid) und bis(Trifluoroacetamid), ZWEI Neue Reagenzien. Zurtrifluoroacetylierung. *J. Chromatogr.* **78**, 273-279.
- Sullivan, J., et al. (1977). Volatile trifluoroacetylated carbohydrates using *N*-Methyl bis(Trifluoroacetamide). *J. Chromatogr. Sci.* **15**, 196-197.
- Weitz, C.J., et al. (1986). Morphine and codeine from mammalian brain. *Proc. Nat. Acad. Sci. USA.* **83**, 9784-9788.

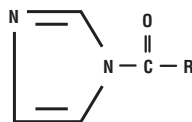
## Ordering Information

Product #	Description	Pkg. Size
✗ 49700	MBTFA [ <i>N</i> -Methyl-bis(trifluoroacetamide)]	10 x 1 ml ampules
✗ 49701	MBTFA	5 g bottle

✗ Additional hazardous handling charge.

# Perfluoroacylimidazoles (TFAI<sup>1-5</sup> and HFBI<sup>6-9</sup>)

**Offer effective acylation of hydroxyl groups and primary and secondary amines.**



R	Name	M.W.	b.p.	d <sub>4</sub> <sup>20</sup>
CF <sub>3</sub>	TFAI	164.08	38-40°C/14 mm	1.490
C <sub>2</sub> F <sub>7</sub>	HFBI	264.10	57-58°C/10 mm	1.562

In many cases, the use of *N*-acylimidazoles offers considerable advantages over acid chlorides and anhydrides. Advantages include:

- The reaction is smooth and positive, releasing no acids into the system to hydrolyze samples
- The byproduct, imidazole, is relatively inert
- Ideal for FID and ECD techniques
- Derivatives are volatile, despite size of group
- Closely bound fluorines contribute stability

Fluorinated acylimidazoles acylate hydroxyl groups and primary and secondary amines. They react smoothly with indole alkylamines to quantitatively derivatize the indole nitrogen, as well as other functional groups present.<sup>1,2</sup> Fluorinated imidazoles also are used for bifunctional derivatizations and in exchange reactions from the TMS derivative to the HFB derivative. In addition, a study by Ikekawa and colleagues found that O-TMS groups could be exchanged to O-HFB groups by adding HFBI and a small amount of HFB acid directly to the reaction mixture.<sup>3,4</sup>

## PROTOCOL 1

Preparing fluoro acyl derivatives for FID.

1. Place 0.1-2.0 mg sample in a 1.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Add 0.2 ml desired *N*-acylimidazole.
3. Cap vial and heat at 60°C 15-30 minutes, or until reaction is complete. (Moderately and hindered steroids may require 2-6 hours heating.)
4. Analyze by FID gas chromatography.

## PROTOCOL 2

Preparing HFB derivatives of indolealkyl amines using HFBI for FID and ECD techniques.<sup>2</sup>

Milligram-scale Derivatization:

1. Combine 2 mg sample and 0.2 ml HFBI in 3 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Cap vial and heat at 85°C for 1 hour.
3. Add 1 ml water and 2 ml toluene.
4. Cap vial and shake 2 minutes.
5. Analyze toluene layer by gas chromatography.

**NOTE:** A small amount of HFB acid remains in toluene phase. If it interferes with analysis, wash toluene phase with 2-3 more 0.5 ml water washes.

Microgram-Scale Derivatization:

1. Combine 2 µg to pg quantities residue and 20 µl HFBI in 3.0 ml Reacti-Vial™ Small Reaction Vial.
2. Cap vial and heat at 85°C for 1 hour.
3. Add 2 ml pure toluene and 0.5 ml distilled water.
4. Cap vial and shake 2 minutes.
5. Remove aqueous layer.
6. Wash toluene layer 3 times with 0.5 ml water.
7. Centrifuge toluene layer 2 minutes.
8. Analyze toluene layer by GC using ECD.

## References

1. Seeley, S.D. and Powell, L.D. (1974). *Anal. Biochem.* **58**, 39-46.
2. Bennington, F., *et al.* (1975). *J. Chromatogr.* **106**, 435-439.
3. Ikekawa, N., *et al.* (1972). *J. Chrom. Sci.* **10**, 233-242.
4. Miyazaki, H., *et al.* (1973). *Anal. Chem.* **45**(7), 1164-1168.
5. Horning, M.G., *et al.* (1968). *Anal. Lett.* **1**, 311-321.
6. Mayhew, J.W., *et al.* (1978). Gas-liquid chromatographic method for the assay of aminoglycoside antibiotics in serum. *J. Chromatogr.* **151**, 133-146.
7. Cohen, H., *et al.* (1984). Capillary gas chromatographic determination of T-2 toxin, HT-2 toxin and diacetoxyscirpenol in cereal grains. *J. Ass. Off Anal. Chem.* **67**(6).
8. Krishnamurthy, T., *et al.* (1987). Mass spectral investigations of trichothecene mycotoxins. II. Detection and quantitation of macrocyclic trichothecenes by gas chromatography/negative ion chemical ionization mass spectrometry. *J. Ass. Off Anal. Chem.* **70**(1).
9. Quilliam, M.A., *et al.* (1980). Study of rearrangement reactions occurring during gas chromatography of *tert*-butyldimethylsilyl ether derivatives of uridine. *J. Chromatogr.* **194**, 379-386.

## Ordering Information

Product #	Description	Pkg. Size
* 48882	TFAI (Trifluoroacetylimidazole)	10 x 1 ml ampules
* 44211	HFBI (Heptafluorobutyrylimidazole)	5 g bottle

\* Additional hazardous handling charge.

\* Additional dry ice and/or freight charges.

# Perfluoro Acid Anhydride

Ours are high-purity, ideal for preparing fluoracyl derivatives.



R	Name	M.W.	b.p.	d <sub>4</sub> <sup>20</sup>
CF <sub>3</sub>	TFAA	210.0	39.5-40.5°C	1.490
C <sub>2</sub> F <sub>5</sub>	PFAA	310.0	74°C	1.571
C <sub>3</sub> F <sub>7</sub>	HFAA	410.0	106-107°C	1.665

Fluorinated anhydrides are used to prepare fluoracyl derivatives for GC/MS, they produce stable volatile derivatives for FID and ECD techniques.

## PROTOCOL 1

Preparing fluoracyl derivatives of amines and alcoholic compounds on a submicrogram scale for ECD.

1. Combine <50 ng sample dissolved in 500 µl benzene and 100 µl 0.05 M TEA in benzene in a 5.0 ml Reacti-Vial™ Small Reaction Vial.
2. Add 10 µl acid anhydride.
3. Cap vial, heat at 50°C 15 minutes, then cool.
4. Add 1 ml water, cap vial and shake 1 minute.
5. Add 1 ml 5% aqueous ammonia, cap vial and shake 5 minutes.
6. Centrifuge.
7. Inject 1-10 µl benzene phase for ECD.

**NOTE:** Use 250 µg for FID. Excess TEA is required for quantitative conversion of amines. TEA does cause disturbances in the chromatogram at high EC sensitivity. The benzene used as sample solvent and TEA solvent should be dry, as water will compete for the anhydride during reactions. The amount of anhydride used in the procedure (10 µl) is 25% more than necessary for a complete reaction – even if the 0.5 ml benzene used is water-saturated.

## PROTOCOL 2

Preparing fluoracyl derivatives of phenols for FID and ECD.

For Flame Ionization Detection:

1. Combine 1 mg sample dissolved in 0.5 ml benzene and 200 µl 0.1 M TEA in benzene.
2. Add 25 µl acid anhydride.
3. Cap vial and let react at RT 15 minutes.
4. Add 0.5 ml 1 M phosphate buffer, pH 6.0, and shake 30 seconds.
5. Centrifuge.
6. Separate organic phase. Analyze by GC.

For Electron Capture Detection:

1. Combine 0.5 ml benzene containing the sample and 10 µl TEA in benzene in 5 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Add 10 µl HFAA.
3. Cap vial and let react at RT 10 minutes.
4. Add 0.5 ml 1 M phosphate buffer, pH 6.0, and shake 30 seconds.
5. Centrifuge; analyze 2 µl benzene phase by GC.

**NOTE:** Excess anhydride and acid are removed by the aqueous extraction. No ECD disturbances from the water or other constituents. HFB-esters of phenols are stable in the presence of water (with aqueous phase at pH ≤ 6.0). Alkaline extraction with reagents, such as aqueous ammonia, decomposes the HFB ester. A pH ≤ 6.0 maintains the TEA catalyst in the protonized form. TEA in the unprotonized form will catalyze decomposition of the esters.

## References

1. Walle, T. and Ehrsson, H. (1970). *Acta Pharm. Suecica* **7**, 389-406.
2. Walle, T. and Ehrsson, H. (1971). *Acta Pharm. Suecica* **8**, 27-38.
3. Ehrsson, H., *et al.* (1971). *Acta Pharm. Suecica* **8**, 319-328.
4. Suzuki, S., *et al.* (1983). Rapid screening method for methamphetamine in urine by colour reaction in a Sep-Pak C<sub>18</sub> cartridge. *J. Chromatogr.* **267**, 381-387. (TFAA)
5. Seifert, W.E., *et al.* (1978). Characterization of mixtures of dipeptides by gas chromatography/mass spectrometry. *Anal. Biochem.* **88**, 149-161. (PFAA)
6. Christophersen, A.S., *et al.* (1987). Identification of opiates in urine by capillary column gas chromatography of two different derivatives. *J. Chromatogr.* **422**, 117-124. (PFAA)
7. Mule, S.J., *et al.* (1988). Confirmation of marijuana, cocaine, morphine, codeine, amphetamines, methamphetamines, phenylcyclidine by GC/MS in urine following immuno-assay screening. *J. Anal. Tox.* **12**, 102-107. (PFAA)

## Ordering Information

Product #	Description	Pkg. Size
✗ 67363	TFAA (Trifluoroacetic Acid Anhydride)	100 g bottle
✗ 65193	PFAA (Pentafluoropropionic Acid Anhydride)	10 x 1 ml ampules
✗ 65192	PFAA (Pentafluoropropionic Acid Anhydride)	25 g bottle
✗ 65191	PFAA (Pentafluoropropionic Acid Anhydride)	100 g bottle
✗ 63164	HFAA (Heptafluorobutyric Acid Anhydride)	10 x 1 ml ampules
✗ 63163	HFAA (Heptafluorobutyric Acid Anhydride)	25 g bottle
✗ 63162	HFAA (Heptafluorobutyric Acid Anhydride)	100 g bottle

✗ Additional hazardous handling charge.

Tel: 800-874-3723 or 815-968-0747 • Fax: 815-968-7316 • Internet: [www.piercenet.com](http://www.piercenet.com)

# Introduction to Pierce Alkylation Reagents

When used in derivatization for gas chromatography, alkylation represents the substitution of an active hydrogen by an aliphatic or aliphatic-aromatic<sup>1</sup> (benzyl) group. This technique is used to modify those compounds containing acidic hydrogens, such as carboxylic acids and phenols. The principal chromatographic use of this reaction is the conversion of organic acids into esters, which produce better chromatograms than the free acids.

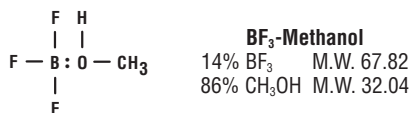
In addition, alkylation reactions can be used to prepare ethers, thioethers and thioesters; *N*-alkylamines; and amides.<sup>2</sup> As the acidity of the active hydrogen decreases, the strength of the alkylating reagent must be increased. As the reagents and conditions become harsher, the selectivity and applicability of the methods become more limited.

## References

1. Kawahara, F.K. (1968). Microdetermination of derivatives of phenols and mercaptans by means of electron capture gas chromatography. *Anal. Chem.* **40**(6), 1009.
2. Kananen, G., *et al.* (1972). Barbiturate analysis – a current assessment. *J. Chrom. Sci.* **10**, 283-287.

## BF<sub>3</sub>-Methanol

**Provides convenient, fast and quantitative esterification of fatty acids.**



BF<sub>3</sub>-Methanol methylation is one of the most convenient ways to prepare methyl esters of fatty acids. Supplied in an easy-to-use septum-sealed Hypo-Vial™ Sample Storage Vial, our BF<sub>3</sub>-Methanol offers convenient syringe removal of your sample – without exposing the contents.

### PROTOCOL 1

For preparing fatty acid methyl esters.

1. Combine 100 mg fatty acid and 3 ml BF<sub>3</sub>-Methanol in a 5.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Cap vial and heat at 60°C for 5-9 minutes.
3. Cool and transfer to separatory funnel with 30 ml hexane.
4. Wash 2 times with saturated NaCl solution.
5. Discard aqueous layers.
6. Dry over sodium sulfate.
7. Evaporate solvent under nitrogen.
8. Analyze by gas chromatography.

### PROTOCOL 2

For preparing C8-C17 fatty acids.

1. Combine 500 mg fatty acid and 5 ml BF<sub>3</sub>-Methanol in a 25 ml flask.
2. Heat on a steam bath 5 minutes.
3. Add saturated NaCl solution until total volume is ~20 ml.
4. Cap flask and invert several times.
5. Allow organic layer to collect at the top, then separate.
6. Dry organic layer over Na<sub>2</sub>SO<sub>4</sub>.
7. Evaporate solvent under nitrogen.
8. Analyze by gas chromatography.

## Reference

1. Yao, Z., *et al.* (1988). Choline deficiency causes translocation of GTP: phosphocholine cytidylyltransferase from cytosol to endoplasmic reticulum in rat liver. *J. Biol. Chem.* **265**(8), 4326-4331.

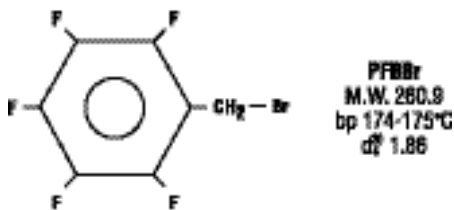
## Ordering Information

Product #	Description	Pkg. Size
✗ 49370	BF <sub>3</sub> -Methanol	100 ml Hypo-Vial™ Sample Storage Vial

✗ Additional hazardous handling charge.

# Pentafluorobenzyl Bromide (PFBBr)

For the electron capture GC analysis of carboxyl acids, phenols and sulfonamides.



Pentafluorobenzylation by an "Extraction Alkylation" technique has been described for the electron capture GC analysis of carboxyl acids, phenols and sulfonamides. This process uses tetrabutylammonium as a counter ion and methylene chloride as a solvent. Reaction times are fast (~20 minutes). Derivatives are highly EC-sensitive, making them useful in low-level determinations of fatty acids.

Kawahara performed extensive work with this reagent, using a potassium carbonate catalyst for the electron capture analysis of mercaptans, phenols and organic acids in surface water.<sup>1-3</sup>

PFBBr has been applied in analyzing trace organics in asphalts, as a "fingerprinting" technique for identifying asphalt pollutants found in surface waters.

## PROTOCOL

For pentafluorobenzylation of acids, phenols and sulfonamides.<sup>1,2</sup>

1. Place 1 ml methylene chloride containing 0.2 mg sample in 3 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Add 1 ml aqueous 0.1 M TBA hydrogen sulfate and 0.2 M sodium hydroxide solution.
3. Add 20 µl PFBBr.
4. Cap vial and shake 20-30 minutes.
5. Inject portion of methylene chloride phase into chromatograph for FID analysis.
6. Evaporate methylene chloride to dryness with nitrogen and redissolve with benzene for ECD analysis.

## References

1. Kawahara, F.K. (1968). *Anal. Chem.* **40**(6), 1009.
2. Kawahara, F.K. (1968). *Anal. Chem.* **40**(13), 2073.
3. Kawahara, F.K. (1971). Gas chromatographic analysis of mercaptans, phenols and organic acids in surface waters with use of pentafluorobenzyl derivatives. *Environ. Sci. Technol.* **5**(3).
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5. Kawahara, F.K. (1976). *Environ. Sci. Technol.* **10**(8), 761.
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8. Lee, H.-B., *et al.* (1984). Chemical derivatization analysis of pesticide residues. IX. Analysis of phenol and 21 chlorinated phenols in natural waters by formation of pentafluorobenzyl ether derivatives. *J. Assoc. Off. Anal. Chem.* **67**(6).
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10. Kok, R.M., *et al.* (1985). Highly sensitive determination of 5-fluorouracil in human plasma by capillary gas chromatography and negative ion chemical ionization mass spectrometry. *J. Chromatogr.* **343**, 59-66.
11. Odham, G., *et al.* (1985). Determination of microbial fatty acid profiles at femtomolar levels in human urine and the initial marine microfouling community by capillary gas chromatography-chemical ionization mass spectrometry with negative ion detection. *J. Microbiol. Meth.* **3**, 331-344.
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15. Chen, S.-H., *et al.* (1987). Simultaneous gas chromatographic determination of iodide, nitrate, sulphide and thiocyanate anions by derivatization with pentafluorobenzyl bromide. *J. Chromatogr.* **396**, 129-137.
16. Bartsch, H., *et al.* (1988). Methods for detecting DNA damaging agents in humans: applications in cancer epidemiology and prevention. *International Agency for Research on Cancer. IARC Scientific Publications* **89**.
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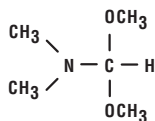
## Ordering Information

Product #	Description	Pkg. Size
✱ 58220	PFBBr (Pentafluorobenzyl Bromide)	5 g

✱ Additional hazardous handling charge.

# Methyl-8<sup>®</sup> Reagent and Concentrate

**For easy, effective preparation of methyl esters from fatty acids and amino acids.**



**Methyl-8<sup>®</sup>**  
 M.W. 119.17  
 bp 102-104°C  
 $d_4^{20}$  0.897

For preparing methyl esters for gas chromatography, our Methyl-8<sup>®</sup> Products offer significant advantages including:

- **Speed** – the reaction is complete upon dissolution
- **Quantitation** – quantitative yields are obtained when reagent and sample are injected – without prior mixing
- **Your choice of formulation** – our Methyl-8<sup>®</sup> Reagent is a convenient, ready-to-use reagent that contains 2 mEq/ml in pyridine. Or you can choose from our Methyl-8<sup>®</sup> Concentrate, which contains no solvent

Methyl-8<sup>®</sup> Reagent is stable at room temperature and is packed in convenient, ready-to-use Hypo-Vial<sup>™</sup> Sample Storage Vials. No water washing, extraction or concentration of the derivatives are required. Plus, no water is formed in the reaction.

Reactions with Methyl-8<sup>®</sup> Reagent usually are complete upon dissolution, except for long chain solid acids. In these applications, it is necessary to use Methyl-8<sup>®</sup> Reagent with additional solvent and mild heating. Suitable solvents include pyridine, benzene, methanol, chloroform, methylene chloride, THF and DMF.

Thenot, *et al.* have demonstrated analytical applications that use Methyl-8<sup>®</sup> Reagent for analyzing fatty acids<sup>1</sup> and amino acids.<sup>2</sup>

## PROTOCOL 1

Methods of alkylation using DMF-Dialkyl Acetal Reagents.

1. Combine 50 mg fatty acid and 1 ml Methyl-8<sup>®</sup> Reagent or 300  $\mu$ l Methyl-8<sup>®</sup> Concentrate in a 3 ml Reacti-Vial<sup>™</sup> Small Reaction Vial (see page 68).
2. Cap vial and heat at 60°C for 10-15 minutes or until dissolution is complete.
3. Analyze by gas chromatography.

## PROTOCOL 2

For preparing *N*-dimethylaminomethylene (DMAM) methyl esters of amino acids.

1. Combine amino acid with 1:1 ratio of Methyl-8<sup>®</sup> Reagent to acetonitrile in a Reacti-Vial<sup>™</sup> Small Reaction Vial (see page 68).
2. Cap vial and heat at 100°C for 20 minutes or until dissolution is complete.
3. Analyze by gas chromatography.

**NOTE:** Aspartic acid requires a longer time for complete dissolution. Hydroxyl groups on hydroxyl-substituted amino acids do not react under the above conditions.

### References

1. Thenot, J.-P., *et al.* (1972). *Anal. Lett.* **5(4)**, 217-233.
2. Thenot, J.-P. and Horning, E.C. (1972). Amino Acid *N*-Dimethylaminomethylene Alkyl Ester. *Anal. Lett.* **5(8)**, 519-529.
3. Zhang, Y., *et al.* (1993). Assay of the acetyl-CoA probe acetyl-sulfamethoxazole and of sulfamethoxazole by gas chromatography-mass spectrometry. *Anal. Biochem.* **212**, 481.

## Ordering Information

Product #	Description	Pkg. Size
49350	<b>Methyl-8<sup>®</sup> Reagent</b> (2 mEq/ml in pyridine) ( <i>N,N</i> -Dimethylformamide dimethyl acetal)	25 ml Hypo-Vial <sup>™</sup> Sample Storage Vial
49356	<b>Methyl-8<sup>®</sup> Concentrate (No Solvent)</b>	10 x 1 ml ampules

# MethElute™ Reagent

**A powerful reagent for accurate, sensitive on-column methylation.**

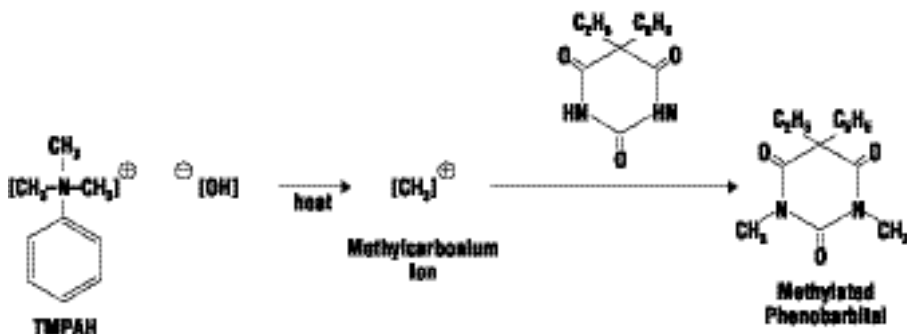


Figure 1. MethElute™ Reagent reaction with phenobarbital.

MethElute™ Reagent [0.2 M trimethylanilinium hydroxide (TMPAH) in methanol solution] is a powerful methylating reagent for quantitative methylation and detection of barbiturates,<sup>1,2,3</sup> sedatives,<sup>1,3</sup> xanthine bases,<sup>2</sup> phenolic alkaloids<sup>2</sup> and Dilantin<sup>3,4</sup> by gas chromatography.

MethElute™ Reagent gives a single quantitative peak response for each substance. When the reagent is heated with drug-containing extracts, serum or urine, those drugs containing reactive amino, hydroxyl and carboxyl functions will be methylated at the reactive sites.

## Performance Characteristics

**Accuracy.** Comparable or better than the UV/TLC method.<sup>5</sup> When phenobarbital and Dilantin are present, the UV/TLC method cannot be used, as Dilantin interferes with the phenobarbital determination. The GC procedure yields excellent results for this combination of drugs.<sup>5</sup>

**Precision.** The coefficient of variation is 5% or less.

**Sensitivity.** Detects barbiturates down to 0.2 mg/dl.<sup>5</sup>

## References

1. *Chemical and Engineering News*, April 12, 1971, page 13.
2. Brochmann-Hansen and Oke, T.O. (1969). *J. Pharm. Sci.* **58**, 370-371.
3. Barrett, M.J. (Spring 1971). *The Clinical Chemistry Newsletter* **3(1)**. Published by the Perkin-Elmer Corp., Norwalk, Conn. 06852.
4. Barrett, M.J. (1970). *Clinical Chemistry Application Study No. 33*. Published by the Perkin-Elmer Corp., Norwalk, Conn. 06852.
5. Kananen, G., et al. (1972). *J. Chromatogr. Sci.* **10**, 283-287.

## Ordering Information

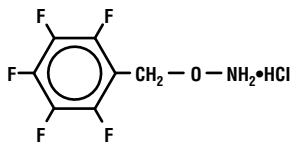
Product #	Description	Pkg. Size
✘ 49300	MethElute™ Reagent	10 ml Hypo-Vial™ Sample Storage Vial
✘ 49301	MethElute™ Reagent	12 x 1 ml Hypo-Vial™ Sample Storage Vials

✘ Additional hazardous handling charge.

# More Specialized Reagents for Your More Specialized Applications

## FLOROX™ Reagent

Offers low-level ECD GC determinations of ketosteroids.<sup>1,2</sup>



FLOROX™ Reagent

M.W. 249.57

FLOROX™ Reagent, an *o*-(pentafluorobenzyl) hydroxylamine-HCl reagent, couples a highly EC-sensitive electrophore directly to keto functions. This results in low-level (0.1 ng) determinations of ketosteroids.

### References

1. Nambara, T., *et al.* (1975). CIII. A new type of derivative or electron capture-gas chromatography of ketosteroids. *J. Chromatogr.* **114**, 81-86.
2. Koshy, K.T., *et al.* (1975). *O*-(2,3,4,5,6-Pentafluorobenzyl) hydroxylamine hydrochloride as a sensitive derivatizing agent for the electron capture gas liquid chromatographic analysis of ketosteroids. *J. Chrom. Sci.* **13**.

### PROTOCOL

For FID Detection of 1-10 µg steroid.<sup>1</sup>

1. Add 0.1 ml FLOROX™ Reagent to 1-10 µg ketosteroids isolated as a residue in a 1.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Cap vial and heat at 65°C for 1 hour; allow to cool.
3. Evaporate reaction mixture with nitrogen.
4. Add 0.1 ml cyclohexane, then 0.1 ml water.
5. Cap vial and shake.
6. Remove upper cyclohexane layer for FID-GC.

For EC Detection of 12.5-50 ng steroid.<sup>1</sup>

1. Add 10 µl FLOROX™ Reagent to 12.5-50 ng steroid residue in a 1 ml Reacti-Vial™ Small Reaction Vial.
2. Cap vial and heat at 65°C for 1 hour; allow to cool.
3. Evaporate reaction mixture with nitrogen to remove pyridine.
4. Dissolve residue in 0.5 ml cyclohexane.
5. Wash with 0.5 ml cyclohexane.
6. Transfer cyclohexane layer to another vial; dry by adding anhydrous Na<sub>2</sub>SO<sub>4</sub>.
7. Use cyclohexane phase for EC-GC.

For EC Detection of 0.1-5 ng steroid.<sup>1</sup>

1. Add 10 µl EC grade pyridine and 2 µl FLOROX™ Reagent to 0.15 ng steroid residue in a 1 ml glass stoppered tube.
2. Cap tube and heat at 65°C for 1 hour; allow to cool.
3. Evaporate reaction mixture with nitrogen to remove pyridine.
4. Dissolve residue in 25-100 µl EC grade cyclohexane.
5. Wash with an equal volume of 10% HClO<sub>4</sub>.
6. Centrifuge and sample upper layer for EC-GC.

**NOTE:** If desired, underivatized hydroxyl groups may be silylated after the pyridine removal step.<sup>1</sup>

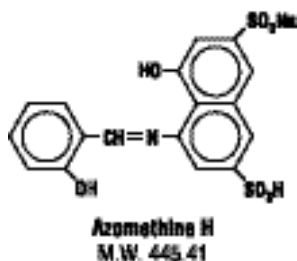
### Ordering Information

Product #	Description	Pkg. Size
✘ 49650	FLOROX™ Reagent Contains 2.5 mg/ml of <i>O</i> -(Pentafluorobenzyl)hydroxylamine•HCl in EC Grade Pyridine	10 ml

✘ Additional hazardous handling charge.

# Azomethine H Boron Reagent

**Provides rapid boron determination by colorimetry.**



Azomethine H Boron Reagent provides fast, reliable and sensitive boron determination in soil, plants, composts, manure, water and nutrient solutions.

## Here's how Azomethine H is reported superior to curcumin and other methods:

- Simplicity – fewer steps are involved in the analysis
- Nonacid system; methods carried in water solution
- More accurate results obtained in plant analysis when compared to spectrographic data
- Essentially free from nitrate interference in soil samples with high nitrate levels
- Starting plant material digest or soil extract can be used to determine other important elements

John, *et al.*<sup>1</sup> studied the effects of temperature, time, concentration and composition of the reaction and reagent mixtures. The result is an improved method for the colorimetric determination of boron in soils and plants.<sup>2</sup>

In addition, Gaines and Mitchell showed that the Azomethine H colorimetric boron method gave a certified value of 33 ppm for NBS orchard leaf reference material No. 1571.<sup>3</sup> This is significant because, for the first time, a colorimetric method was given the certified value of an NBS standard. These certified values are otherwise determined with much more sophisticated analytical instrumentation, such as isotope dilution mass spectrometry, nuclear track technique and optical emission spectroscopy.

### References

1. Basson, W.D., *et al.* (1969). *Analyst* **94**, 1135-1141.
2. John, M.K., *et al.* (1975). *Anal. Lett.* **8(8)**, 559-568.
3. Gaines, T.P. and Mitchell, G.A. (1979). *Comm. in Soil Sci. and Plant Anal.* **10(8)**, 1099-1108.
4. Wolf, B. (1971). The determination of boron in soil extracts, plant materials, composts, manures, water and nutrient solutions. *Comm. in Soil Sci. and Plant Anal.* **2(5)**, 363-374.
5. DiLorenzo, A. (1973). Quantitative gas chromatographic analysis of boron trichloride, boron and boron nitride. *J. Chromatogr.* **75**, 207-212.
6. White, C.E., *et al.* (1947). *Anal. Chem.* **19**, 802.

## Ordering Information

Product #	Description	Pkg. Size
40893	Azomethine H Boron Reagent	25 g

# Pierce Multifunctional Silanes/ Surface Treatment Reagents

## An easy way to bond polymer films to surfaces.

Pierce Multifunctional Siliconizing Fluids are specially designed to chemically bind microscopically thin, water-repellent films to glass, quartz, silica and ceramics. The coated surfaces are neutral, hydrophobic and non-oily. In addition, they offer increased resistivity and are not affected by solvents that are not readily hydrolyzed.

### Use Pierce AquaSil™ or SurfaSil™ Siliconizing Fluids to treat pipettes, beakers, certain plastics, ceramics, fiber optics and more:

- For clean drainage and elimination of meniscus
- To reduce adsorption of polar compounds, proteins and trace metals onto glass surfaces and reduce leaching of trace metals into solution

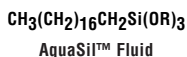
- To prevent current tracking and minimize electrical leakage on glass surfaces and ceramics
- To protect delicate samples against the possible reactive effects of -OH sites present on all types of glassware

### More reasons to use Pierce AquaSil™ and SurfaSil™ Siliconizing Fluids:

- Easy to apply
- Economical – a little bit goes a long way
- Surfaces can easily be recoated
- Improve surface water-repellency
- Increase surface resistivity
- Excellent lubricity
- Non-oily

## AquaSil™ Siliconizing Fluid

### Our water-soluble fluid for siliconizing glass surfaces.



AquaSil™ Siliconizing Fluid is an easy-to-use silane monomer solution that is supplied as a 20% solid solution in a mixture of diacetone alcohol and tertiary butyl alcohol. The primary silane component is an octadecyltrialkoxysilane that, when mixed with water, is hydrolyzed to a silanol. This silanol condenses with available hydroxyl groups and other silane monomers to form a film on the glass surface.

AquaSil™ Siliconizing Fluid is especially useful in the biochemical field because of its aqueous phase application to glass and its greater resistance to base hydrolysis.

### Instructions for Use

AquaSil™ Siliconizing Fluid is applied to a clean glass surface as a dilute aqueous solution. Prepare a 0.1-1.0% solution by weight or volume.

[**Note:** AquaSil™ Fluid contains 20% solids; therefore, one part AquaSil™ Fluid plus 99 parts water (w/w) yields a 0.2% solution – not 1%.] Add the AquaSil™ Fluid to water with constant stirring. A clear-to-slightly hazy solution will be obtained. Solutions are not indefinitely stable and will turn cloudy and precipitate after several days and, therefore, solution should be prepared just before use. Solution stability can be optimized, however, by adjusting the aqueous solution pH to 4.5-5.0.

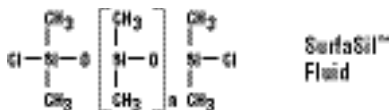
The article to be coated is dipped into the solution, or the surface is flooded with the solution. A thin film will immediately lubricate the glass surface, making it water-repellent. The surface then is air-dried for 24 hours or heated at 100°C for several minutes. Exact drying conditions should be determined before use in commercial process applications.

## Ordering Information

Product #	Description	Pkg. Size
✘ 42799	AquaSil™ Siliconizing Fluid	120 ml
✘ Additional hazardous handling charge.		

# SurfaSil™ Siliconizing Fluid

**A hydrocarbon soluble fluid for siliconizing glass surfaces.**



SurfaSil™ Siliconizing Fluid is a short chain, clear polymeric silicone fluid consisting primarily of dichlorooctamethyltetrasiloxane. When applied to glass, quartz or similar products, the unhydrolyzed chlorines present on the chain react with surface silanols to form a neutral, hydrophobic and tightly bonded film over the entire surface.

SurfaSil™ Siliconizing Fluid is ideal for use on metals, certain plastics, ceramics and fiber optics.

SurfaSil™ Fluid is acidic and care should be taken to avoid corrosion of metal that comes into contact with the liquid. The fluid is acidic only during application. After application the surface is neutral.

**Caution:** Material is flammable before film is formed and HCl fumes are generated in the application.

## Instructions for Use

**Wipe-on treatment:** Wearing rubber gloves, wet a cloth with undiluted fluid and rub it on the clean surface until an oily film is formed. Rub with a dry cloth until the surface is clear.

**Solution treatment:** Dilute SurfaSil™ Fluid with 1-10% clean dry solvent such as acetone, toluene, carbon tetrachloride, methylene chloride or hexane. Do not use esters or alcohols. Articles can be dipped and air-dried. No heating is required. For a slightly more durable coating, heat articles at 100°C for 30 minutes.

SurfaSil™ Siliconizing Fluid (as supplied) is stable for at least one year. Discard prepared solutions after use.

Jevons, *et al.* reported treating all glassware with a 10% v/v solution of SurfaSil™ Fluid in carbon tetrachloride.<sup>1</sup>

## References

1. Jevons, S., *et al.* (1971). Biochemistry of blood platelets. Interaction of activated Factor X with platelets. *Biochemistry*, **10**(3), 428-434.
2. Gershenson, J., *et al.* (1987). Mechanized techniques for the selective extraction of enzymes from plant epidermal glands. *Anal. Biochem.* **163**, 159-164.

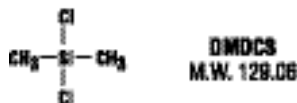
## Ordering Information

Product #	Description	Pkg. Size
✗ 42800	SurfaSil™ Siliconizing Fluid	120 ml
✗ 42801	SurfaSil™ Siliconizing Fluid	480 ml
✗ 42855	SurfaSil™ Siliconizing Fluid	5 x 10 ml ampules

✗ Additional hazardous handling charge.

## DMDCS

**A more thermally stable way to deactivate polar hydroxyls on glass surfaces.**



Coating with Pierce DMDCS is thermally more stable than those achieved with AquaSil™ or SurfaSil™ Fluids. Use full-strength, or as a 10%

solution in an unreactive solvent such as toluene, hexane or methylene chloride. Simply pour reagent through columns or dip article and follow with a methanol rinse. Blow dry with N<sub>2</sub> or air.

## Reference

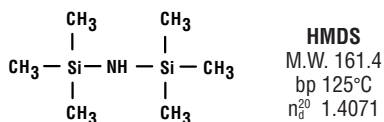
1. Baykut, S., *et al.* (1981). Investigation of physico-chemical behavior of trimethylsilyl derivatives of asymmetric DL-β-hydroxy-β-β'-dialkyl propionic acids on SE 30 liquid phase by gas liquid chromatography. *Chimica Acta Turcia* **9**.

## Ordering Information

Product #	Description	Pkg. Size
83410	DMDCS (Dimethyldichlorosilane)	100 g

# Monofunctional Silane - HMDS

**Ideal for active surface site deactivation.**



HMDS is a popular monofunctional silane that many researchers have found useful for deactivating and coating chromatographic supports. Because of its monofunctional nature, this silane can react with only one site on the surface. Polymerization is not possible, eliminating the chances for unbound polymers to float free and elute from the column – avoiding exposure of unreacted silanols beneath the layer. In addition, surface moisture is eliminated because monofunctional reagents dehydrate the surface.

There are several methods for deactivating surfaces with HMDS:

1. Slurrying or dipping the items to be deactivated in a 5-10% solution of the reagent in an unreactive solvent.
2. Vapor phase deactivating by pulling straight vapor into an evacuated container that holds the item to be deactivated.
3. Placing the item and a few milliliters of the reagent in a beaker, then placing a watch glass on top (as in the case of glass wool silanization).

#### References

1. Nawrocki, J. (1985). Modification of silica with mixture, at hexamethylcyclotrisilazane. *Chromatographia* **20**(5).
2. Owens, N.F., *et al.* (1987). Inhibition of cell adhesion by a synthetic polymer absorbed to glass shown under defined hydrodynamic stress. *J. Cell Sci.* **87**, 667-675.

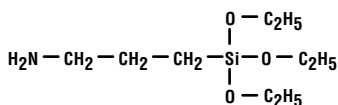
## Ordering Information

Product #	Description	Pkg. Size
✖ 84769	<b>HMDS</b> (Hexamethyldisilazane)	100 g

✖ Additional hazardous handling charge.

# 3-Aminopropyltriethoxysilane

**Useful for preparing amino functional surfaces on glass and silica.**



**3-Aminopropyltriethoxysilane**  
M.W. 221.37

This monofunctional silane is used for chemically coupling various ligands to glass or silica. The reagent first is coupled to glass or silica. Compounds of interest are coupled to the amino groups directly, or additional chemistries are applied using the amino function before coupling.

#### Instructions for Use

1. Dilute reagent to 20% with acetone.
2. Immerse glass.

## Ordering Information

Product #	Description	Pkg. Size
✖ 80370	<b>3-Aminopropyltriethoxysilane</b>	100 g

✖ Additional hazardous handling charge.

# Pierce Column Conditioners Keep GC Columns at Peak Efficiency

## Silyl-8™ GC Column Conditioner

**Injects directly into chromatograph for fast, easy column conditioning.**

### Highlights:

- Easy to use
- Extends column life
- Quickly restores column to peak efficiency
- Improves resolution, reducing tailing
- Helps prevent breakdown of sensitive compounds
- Removes silylatable residues that contaminate chromatographic systems

Silyl-8™ Reagent conditions GC columns by blocking active sites. It has no unexpected side effects and does not release corrosive vapors. Frequent use of Silyl-8™ Conditioner enhances column performance and extends column life.

Pierce Silyl-8™ GC Column Conditioner is available in 25 ml Hypo-Vial™ Sample Storage Vials. These elastomer-capped containers allow immediate access to the reagent – without exposing the sensitive ingredients. In addition, these unique

vials add economy to your research by preventing reagent evaporation.

### Instructions

Inject 10-50 µl Silyl-8™ Conditioner directly into chromatograph with column temperature at 150-250°C (170-200°C is ideal). Conditioning is complete at 175°C in 2-3 minutes. If a stable baseline is not attained with the first attempt, treatment may be repeated until baseline is stabilized. Silyl-8™ GC Column Conditioner may be used as often as necessary without harming the bonded capillary column.

**NOTE:** Silyl-8™ Conditioner should not be used on H-bonding type phases such as glycerols or Carbowax® Glycols. In addition, it should not be used on phases that depend on reactive hydrogens (such as -OH, -SH, -COOH, -HN<sub>2</sub> or =NH) for their function.

## Ordering Information

Product #	Description	Pkg. Size
✘ 38015	Silyl-8™ GC Column Conditioner	25 ml Hypo-Vial™ Sample Storage Vial

✘ Additional hazardous handling charge.

## Books

## Handbook of Analytical Derivatization Reaction

**A self-contained methodology reference manual and efficient entry point to the original literature resource book.**



*The Handbook of Analytical Derivatization Reactions* by Daniel R. Knapp is a general collection of analytical derivatization methods for chromatography and mass spectroscopy involving the formation of covalent derivatives before analysis.

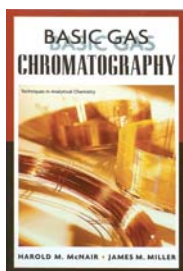
Methods contained in this volume are organized according to the type of sample being derivatized. Methods include structural formulas, experimental directions and useful comments. A thorough system of indexing takes you quickly to the “lab ready” methods of interest.

### Ordering Information

Product #	Description
15012	<b>Handbook of Analytical Derivatization Reactions</b> Knapp, D.R. Ed (1979) Published by John Wiley and Sons, Inc. Hardcover, 741 pages

## Basic Gas Chromatography

**Provides up-to-date information on the use of capillary and packed GC column techniques.**



This book is written for readers with a variety of educational and laboratory backgrounds.

Written by two prominent GC experts, this book contains topics on chiral separations, GC/MS, solid-phase microextraction, derivatization, headspace techniques, and a helpful troubleshooting section. A list of suppliers, references and applications will help you establish your own GC analysis laboratory.

### Ordering Information

Product #	Description
15003	<b>Basic Gas Chromatography</b> McNair, H.H. and Miller, J.M. Ed (1998) Published by John Wiley and Sons, Inc. Hardcover, 224 pages



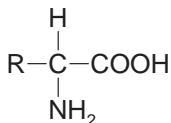
# Amino Acid Analysis

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# Amino Acid Analysis — The History

The development of amino acid analysis began in 1820 when Braconnot isolated glycine from a hydrolyzate of gelatin.<sup>1</sup> Later, in 1848, the Dutch chemist Mulder showed that glycine contains nitrogen, a major component of amino acids.<sup>2</sup> It was not until 1883, however, that Kjeldahl introduced a method that accurately determined the amount of nitrogen in a protein/amino acid sample.<sup>3</sup>

By 1910, most of the amino acids had been isolated and their structures discovered. As the number of known amino acids accumulated, it became possible to group them on the basis of common chemical features. At that time, it was noticed that all amino acids have the same general formula and differ only by the chemical structure of the side chains.



From 1910-1940, amino acid research was characterized by the work of quantitative analysts, as opposed to the organic chemists of the 1800s. Amino acid analyses conducted during the 1800s and early 1900s were laborious, often extending over weeks and months. While the amino acid content of a number of proteins was discovered, exact information was not always obtainable using the equipment available during that time period.<sup>4</sup>

The introduction of chromatography opened new doors for amino acid analysis. The first breakthrough came when Martin and Syngue introduced partition chromatography, which separates the acetyl derivatives of certain amino acids.<sup>5</sup> In this method, an equilibrium is established between two liquid phases. Silica gel is mixed with a solution of water and an indicator. The resulting slurry is packed into a column, forming the stationary phase. Next, the acetyl amino acids are dissolved in solvent, forming the mobile phase. The acids then are placed in the same column. The acetyl

amino acids flow through the column at different rates. Separation is made visible by the bands of color change in the indicator.

While this system successfully separated mono-amino and mono-carboxylic acids, it was impractical for other types of amino acids.

Later, Martin and his associates used filter paper as an alternative to silica gel, developing a paper chromatography method that is still in use today. The amino acids were dissolved in butanol and allowed to seep onto the filter paper for a set amount of time. The paper then was dried and sprayed with a dilute solution of ninhydrin (2,2-dihydroxy-1,3-indandione) in butanol. The colored spots were measured and compared with the set values for those experimental conditions.

The separations achieved with paper chromatography were only semi-quantitative. Column chromatography, on the other hand, had potential for quantitation, but the separations were imperfect. The introduction of ion exchange chromatography solved these problems, allowing column separation of amino acids without any prior derivatization.<sup>6,7</sup> Initially used to remove carbohydrate contamination from starch columns, ion exchange resins were quickly found to have great potential for separating amino acids. While many types of polymeric exchange resins were tested, polysulfonic resins (such as Dowex 50) provided the best separations.<sup>8</sup>

Modifications to these procedures have improved amino acid separations. Resin characteristics, column size, column temperature, buffer pH and ionic strength all have been modified to improve resolution of amino acid mixtures and achieve specific separations. Also, quantitation was greatly improved by the use of post-column reactions with ninhydrin. At one time ninhydrin was the most widely used detection system; however, more sensitive indicators, such as *o*-phthalaldehyde were developed to increase analytical sensitivity.

### Developments in Amino Acid Analysis

Improvements in amino acid analysis by ion exchange chromatography have involved the analytical system, as well as the instrumentation. Systems have been developed (by varying buffer pH or ionic strength) that work to displace the amino acids into discrete bands. The buffer systems are compatible with single- or two-column analysis of amino acids found in protein hydrolyzates or physiological fluids. Buffer systems are determined by the counter ion used (sodium or lithium) and by the method of buffer changes introduced to the resin (step changes or gradient elution).<sup>9-15</sup> The most commonly used buffering component, citrate, is suitable for solutions below pH 7.<sup>16</sup> Buffers are prepared either with citric acid or an alkali salt, and citrate concentrations of 0.05 to 0.06 M are common.

Unfortunately, for high-sensitivity work, citric acid is a significant contributor to amino acid contamination. Therefore, to achieve consistent analyses, it is essential to use high-purity reagents for buffer preparation.

Alternatives to ion exchange are emerging for the separation of amino acids. Because amino acid analysis is one of the basic protein chemistry tools available, more rapid and sensitive methods for separation and quantitation are desirable.<sup>17</sup> Several pre-column derivatization methods using reverse-phase HPLC have been developed.

Two of the most widely used of these methods involve the formation of dansyl<sup>18-19</sup> or *o*-phthalaldehyde (OPA)<sup>20-24</sup> derivatives of amino acids

prior to HPLC analysis. Both methods offer greater sensitivity and shorter analysis time than post-column derivatization techniques. Other methods include the quantitative derivatization of amino acids with phenylisothiocyanate (PITC) and the separation and quantitation of the resulting phenylthiocarbonyl derivatives via HPLC. These derivatives are stable enough to eliminate in-line derivatization.

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# Introduction to Sample Preparation and Hydrolysis Methods

The extraction and purification of proteins play an important role in determining amino acid content. These methods are based on one or more of their physical characteristics (e.g., solubility, molecular size, charge, polarity and specific covalent or noncovalent interactions). The techniques commonly used to separate proteins and peptides include:

- Reverse-phase HPLC
- Polyacrylamide gel electrophoresis
- Gel filtration
- Ion exchange chromatography
- Affinity chromatography

Table 1 provides a more detailed list of methods for fractionating peptide mixtures.<sup>1</sup>

**Table 1. Methods for the fractionation of peptide mixtures**

Technique	Properties of Peptide Molecules Exploited
Centrifugation	Solubility
Gel filtration	Size
Ion exchange chromatography	Charge, with some influence of polarity
Paper electrophoresis	Charge and size
Paper chromatography	Polarity
Thin layer electrophoresis	Charge and size
Thin layer chromatography	Polarity
Polyacrylamide gel electrophoresis	Charge and size
High-performance liquid chromatography (HPLC)	Polarity
Gas chromatography	Volatility of derivatives
Counter-current extraction	Polarity; sometimes specific interactions
Affinity chromatography	Specific interactions
Covalent chromatography or irreversible binding	Disulfide bond formation; reactivity of homoserine lactone

## Hydrolysis

Most protein samples require some form of chemical treatment before their component amino acids are suitable for analysis. Protein and peptide samples must be hydrolyzed to free amino acids from peptide linkages. Acids (usually HCl) are the most widely used agents for hydrolyzing proteins.

A simplified hydrolysis procedure involves refluxing the protein with excess HCl, then removing the excess acid in vacuum.<sup>2</sup> The lyophilized protein then is suspended in 6 N HCl and introduced into the hydrolysis tube. The sample is frozen by immersing the tube in dry ice and acetone. Before sealing, the tube is evacuated to avoid formation of cysteic acid, methionine sulfoxide and chloroty-

rosine.<sup>3</sup> This procedure minimizes decomposition of reduced S-carboxymethylcysteine and analyzes S-carboxymethylated proteins. Hydrolysis is conducted at 110°C (with the temperature accurately controlled) for 20-70 hours by Moore and Stein's method.<sup>4</sup> After hydrolysis, residual HCl is removed in a rotary evaporator. The residue is dissolved in water and brought to the appropriate pH for addition to the analyzer column.<sup>4</sup>

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4. Moore and Stein, *op. cit.*

## PROTOCOL

### A. Preparation

1. Unscrew the Teflon® Plug from the 5 ml vacuum hydrolysis tube.
2. Using a small diameter glass pipette (disposable Pasteur) or syringe, introduce the sample into the bottom reservoir of the tube. Avoid leaving any residue of the sample in the upper portion of the tube, particularly in the area of the threads, and at the top of the reservoir where the Teflon® Plug makes a seal.
3. Add the specially purified constant boiling hydrochloric acid up to the desired reaction level.

**NOTE:** For best results, the total volume of sample and hydrochloric acid should not exceed 1/3 of the stated reservoir volume. This will help prevent loss during the application of vacuum, when bumping and foaming may occur. To prevent excessive bumping and foaming during the vacuum process, the sample can be frozen prior to the application of vacuum.

### B. Application of Vacuum

1. Insert the inert Teflon® Plug and screw it down just enough to leave a small passageway between the plug and the glass at the constricture point.
2. Secure the tube and affix the vacuum source to the side arm. It is helpful to use vacuum tubing to connect the hydrolysis tubes to a three-way stopcock. The stopcock should be connected to a vacuum source and a supply of purified nitrogen or argon. This will allow you to alternate between vacuum and an inert gas, which ensures the complete removal of oxygen from the sample. Additionally, this set-up will facilitate the controlled release of the vacuum after the hydrolysis is complete.

### C. Hydrolysis

1. When the desired vacuum is reached, seal the unit by slowly screwing the plug down until it is flush with the glass surface at the constricture. **Do not overtighten!** Applying too much pressure to the hydrolysis tube is the most common cause of damage to the tube or Teflon® Plug. Pierce Vacuum Hydrolysis Tubes are designed to withstand temperatures up to 200°C for 48 hours.

**NOTE:** At 150°C, specially purified hydrochloric acid allows rapid (6-hour) hydrolysis of proteins. (Standard protein hydrolysis conditions are 105-110°C for 16-24 hours.)

2. After the tubes have cooled, release the Teflon® Plug just enough to create a small passageway between the plug and the glass constricture. The three-way stopcock set-up, described earlier, facilitates this procedure and allows you to break the vacuum without allowing air/oxygen into your sample.

### D. Sample Concentration

1. After securely fastening the tube, your sample can be concentrated (via vacuum) with or without heating the tube. Alternatively, the hydrolysis reagent may be removed by lyophilization, or via a stream of nitrogen/argon directed to the surface of the sample.
2. At the appropriate point in your procedure, use a small diameter pipette to remove your sample. The hydrolyzed sample can easily be redissolved in the sodium citrate sample dilution buffer.
3. Completely remove the Teflon® Plug and use a small pipette to add the appropriate quantity of buffer to the dried sample.
4. Allow the sample to dissolve and remove it with a pipette.

# Vacuum Reaction System

**Featuring our Reacti-Therm™ Heating Modules – the only dry block heating systems custom-designed for vacuum reaction tubes.**



Pierce Reacti-Therm™ Dry Block Heating Modules feature everything you need for efficient protein hydrolysis. Precise temperature control, uniform heating and an expanded temperature range make these units an effective and economical hydrolysis choice. They are ruggedly built and feature separate high/low thermostats. A convenient blinker light signals when temperature is stabilized.

The Reacti-Therm™ Heating Modules incorporate interchangeable Reacti-Block™ Aluminum Blocks, which accommodate all sizes of vacuum reaction tubes. These unique heating modules are available in two sizes – one is sure to meet your sample needs.

## **Reacti-Therm™ Heating Module – great for small sample capacity applications:**

- Accommodates a single Reacti-Block™ Aluminum Block (see page 44)
- Temperature range ambient to 150°C
- Temperature controlled  $\pm 0.5^\circ\text{C}$
- Complete instructions included

## **Reacti-Therm™ III Heating Module – three times the sample capacity!**

- Accommodates three Reacti-Block™ Aluminum Blocks (see page 44) for larger capacity requirements
- Temperature range ambient to 200°C
- Temperature controlled  $\pm 0.5^\circ\text{C}$  at 375°C for incubation
- Complete instructions included

## Ordering Information

Product #	Description	Voltage
18870	Reacti-Therm™ (Single Block) Heating Module	110 v
18835	Reacti-Therm™ III (Triple Block) Heating Module	110 v
18790	Reacti-Therm™ (Single Block) Heating Module	220 v
18840	Reacti-Therm™ III (Triple Block) Heating Module	220 v

*Underwriters Laboratories, Inc. Listed*

*Note: Our 220-volt modules bear a CE marking for meeting the requirements of the European Union's Low-Voltage and EMC Directives. Refer to page 79-80 for appropriate blocks.*

# Vacuum Reaction Tubes

Use with our Reacti-Therm™ System for fast, effective protein hydrolysis.



Pierce reusable Vacuum Reaction Tubes are ideal for:

- Hydrolysis
- Sample concentration
- Lyophilization
- Hydrazinolysis

Here's how easy it is to create a vacuum seal with our Vacuum Reaction Tubes:

1. Insert sample into bottom of vacuum hydrolysis tube. Sample should not exceed 1/3 of the volume of the tube from the constriction down.
2. Screw Teflon® Plug down, leaving room at the base of the plug for air to pass.
3. Use a vacuum pump to draw a vacuum through the side arm, and slowly screw the plug down until the plunger is flush with the glass surface of the constriction. **Do not overtighten!**
4. The sample is ready for heating. Do not heat the vacuum hydrolysis tube to greater than 110°C in an oven, because the Teflon® Plug is not rated to withstand greater temperatures. Use a Reacti-Therm™ Dry Block Heating System for optimal control of your hydrolysis and better care of your hydrolysis tubes. When used in the appropriate Reacti-Therm™ System, Pierce Vacuum Hydrolysis Tubes are designed to withstand temperatures of 150°C\* for six-hour hydrolysis procedures.

*\* The upper temperature limit of the Vacuum Hydrolysis Tubes is 260°C.*

## Ordering Information

Product #	Description	Pkg. Size
29550	Vacuum Hydrolysis Tube	8 mm x 60 mm, 1 ml volume
29560	Vacuum Hydrolysis Tube	10 mm x 100 mm, 5 ml volume
29564	Vacuum Hydrolysis Tube	19 mm x 100 mm, 20 ml volume

# Reacti-Block™ Aluminum Blocks

**Accommodate all sizes of Vacuum Reaction Tubes for efficient protein hydrolysis with our Reacti-Therm™ System.**



Reacti-Block™ Aluminum Blocks are precisely machined and drilled for use in our Reacti-Therm™ Heating Modules. Each block contains a thermometer well (9/32" dia. x 1-7/16" deep) and holes that accept our Vacuum Reaction Tubes. When block temperature is stabilized, it is approximately 5°C higher than the solution in the tubes.

**Reacti-Block™ F:** Holds eight Vacuum Reaction Tubes; eight holes – 10 mm dia. x 64 deep

**Reacti-Block™ G:** Holds four Vacuum Reaction Tubes; four holes – 19 mm dia. x 64 mm deep

## Ordering Information

Product #	Description	Note
18806	Reacti-Block™ F	Accepts tube No. 29560
18807	Reacti-Block™ G	Accepts tube No. 29564

# Our Ready-to-Use Hydrolysis Reagents

Pierce Hydrolysis Reagents are purified and packaged to guarantee a ninhydrin negative blank on hydrolysis. Convenient pre-scored ampule

packaging eliminates contamination that can result from exposure to lab atmospheres and repeated use of stock solutions.

## Constant Boiling (6N) Hydrochloric Acid

### For total protein hydrolysis.

Eveleigh and Winter give an excellent description of the total protein hydrolysis technique using Constant Boiling Hydrochloric Acid.<sup>1</sup> Standard protein hydrolysis conditions are 105-110°C for 16-24 hours. At 150°C, this reagent can hydrolyze peptides in 6 hours.

### References

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## Ordering Information

Product #	Description	Pkg. Size
✗ 24308	Hydrochloric Acid [Constant boiling (6N), Sequanal Grade]	10 x 1 ml ampules

✗ Additional hazardous handling charge.

# Amino Acid Standard H

**Our high-purity amino acid calibration standard for protein hydrolyzates.**

The high-purity amino acids of Standard H are ideal for calibrating amino acid analyzers. To permit standardization of microbiological and other assays, we have used the L-form configuration. The molar concentration of each standard is verified by conventional amino acid analysis methods.

With the exception of cystine, each amino acid is supplied at a concentration of 2.5  $\mu\text{moles/ml}$  in 0.1 N HCl. The following amino acids are included with our Amino Acid Standard H:

L-Alanine	L-Leucine
Ammonia $[(\text{NH}_4)_2 \text{SO}_4]$	L-Lysine•HCl
L-Arginine	L-Methionine
L-Aspartic Acid	L-Phenylalanine
L-Cystine	L-Proline
L-Glutamic Acid	L-Serine
Glycine	L-Threonine
L-Histidine	L-Tyrosine
L-Isoleucine	L-Valine

## Instructions for Use

Thaw Standard H and shake well. Dilute appropriately with suitable buffers to a concentration compatible with the full-scale sensitivity of your amino acid analyzer.

## Storage

When kept frozen, an unopened vial has an indefinite storage life. Once the seal is broken, the reagent has a maximum storage life of six months. Store Amino Acid Standard H frozen between uses.

## Ordering Information

Product #	Description	Pkg. Size
20088	Amino Acid Standard H	10 x 1 ml ampules

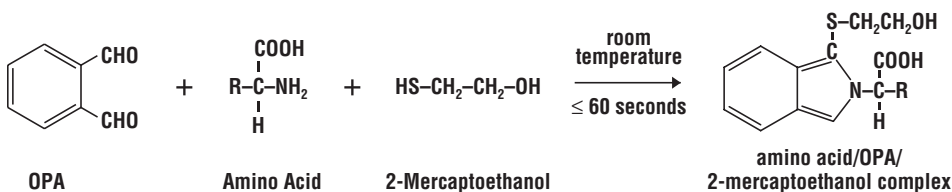
# Introduction to Fluorometric Detection of Amino Acids

## *o*-Phthalaldehyde Detection of Amino Acids and Primary Amines

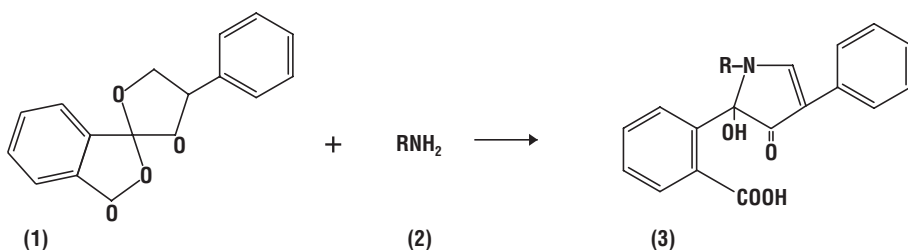
Ion exchange chromatography of amino acids, followed by post-column reaction with the highly sensitive *o*-phthalaldehyde (OPA) fluorophoric reagent, allows measurement of amino acids at the sub-nanomole level.<sup>1-8</sup>

High-sensitivity detection is achieved by post-column reaction of amino acids with OPA/2-mercaptoethanol in a potassium borate buffer. The amino acid/OPA/2-mercaptoethanol reaction complex has an excitation wavelength of 360 nm and fluoresces at 455 nm. This reaction is complete in less than one minute at room temperature.<sup>2</sup>

The OPA technique has been reported to be 10 times more sensitive than the ninhydrin reaction. Detection of secondary amines, such as proline and hydroxyproline, is not possible unless they are first oxidized with a dilute solution of sodium hypochlorite. Under alkaline conditions, sodium hypochlorite converts the secondary amines (imino acids) to primary amines, generating fluorescence after reacting with OPA.<sup>10</sup> Recent improvements,<sup>3-4</sup> along with the adaptation of hypochlorite oxidation for proline analysis,<sup>8,10-12</sup> makes the OPA/2-mercaptoethanol technique suitable for routine analysis.



**Reaction Scheme 1.** *o*-Phthalaldehyde reaction with an amino acid in presence of 2-mercaptoethanol. Reaction occurs quickly at room temperature to form an amino acid/OPA/2-mercaptoethanol fluorescent complex.



**Reaction Scheme 2.** Fluorescamine (1) reacts with primary amines (2), forming intensely fluorescent substances (3).

### Fluorescamine Detection of Amino Acids and Primary Amines

Fluorescamine reacts rapidly with primary amines at room temperature. The fluorophores produced have an excitation wavelength of 390 nm and an emission wavelength of 475 nm. A detailed discussion of the fluorescamine reaction is found in the paper by Udenfriend, *et al.*<sup>13</sup>

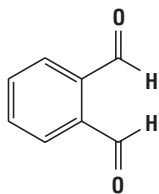
Delivered into suitably buffered column effluents, fluorescamine in an acetone solution has been used in amino acid analysis.<sup>14</sup> Certain secondary amino acids (such as proline and hydroxyproline) first must be oxidized with *N*-chlorosuccinimide. This produces responsive primary amino acids, which can be detected by fluorescamine.<sup>15-18</sup> Reports have compared the sensitivities of fluorescamine and ninhydrin on a microbore system.<sup>19</sup>

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# Fluoraldehyde™ *o*-Phthalaldehyde (OPA) Reagent Solution

Great for pre- or post-column fluorescent detection of amines.<sup>1,2,5</sup>



## Fluoraldehyde™ Reagent Solution (*o*-Phthalaldehyde)

M.W. 134.13

$\lambda_{ex}$  = 340 nm

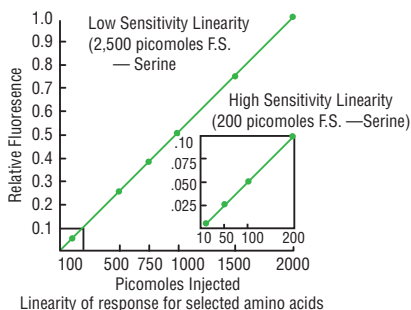
$\lambda_{em}$  = 455 nm

Pierce Fluoraldehyde™ Reagent Solution contains a stabilized, highly purified preparation of *o*-phthalaldehyde, Brij®-35 Detergent and mercaptoethanol in a specially formulated borate buffer. It is a highly sensitive, ready-to-use reagent solution that exhibits excellent linear response (Figure 1) and offers outstanding shelf life (Figure 2). In addition, when compared to other *o*-phthalaldehyde detection reagents, our solution exhibits decreased background over time and a high signal-to-noise ratio.

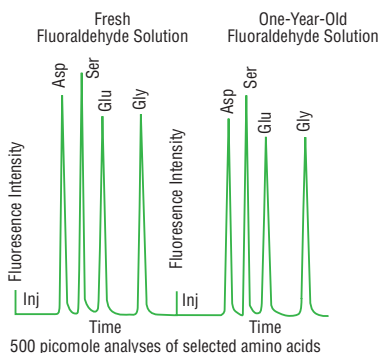
**NOTE:** See page 54 for pre-column protocol using Fluoraldehyde™ OPA Reagent Solution

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**Figure 1. Excellent linear response.** Fluoraldehyde™ Reagent Solution shows excellent linear response, whether in the 2,500 or 200 picomole range.



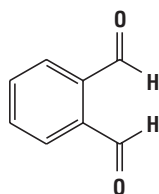
**Figure 2. Outstanding shelf life.** Comparison of fluorescence response of selected amino acids after reaction with recently prepared and one-year-old Fluoraldehyde™ Reagent Solutions.

## Ordering Information

Product #	Description	Pkg. Size
26025	Fluoraldehyde™ Reagent Solution (0.8 mg/ml <i>o</i> -phthalaldehyde)	945 ml

# Fluoraldehyde™ Crystals

An easy-to-use, yet economical way to detect amino acids in pre-<sup>1</sup> and post-column<sup>2,3</sup> chromatographic effluents.



Fluoraldehyde™ Crystals  
(*o*-Phthalaldehyde)  
M.W. 134.13

Pierce Fluoraldehyde™ Crystals are stable in aqueous solution, making them a highly sensitive, yet economical and easy-to-use purified grade of crystalline *o*-phthalaldehyde. While no heating is required with Fluoraldehyde™ Crystals, they allow rapid analysis and exhibit low background.

A common procedure involves dissolving Fluoraldehyde™ Crystals in a pH 10.4 potassium borate buffer solution for a final concentration of 0.8 mg/ml.

## Applications

- Post-column cysteine and cystine derivatization<sup>2</sup>
- Automated pre-column derivatization of plasma amino acids<sup>3</sup>
- Pre-column derivatization of free physiological amino acids in tissues and biological fluids<sup>4</sup>
- Derivatization of amino acids from biopsy specimens. Comparison of OPA reagents using  $\beta$ -mercaptoethanol, ethane thiol or mercaptopropionic acid shows mercaptopropionic acid derivatives most stable<sup>5</sup>
- Pre-column optimized for low picomole amounts<sup>6</sup>
- Optimized reaction conditions and studied on-column stability of OPA-AA derivatives<sup>7</sup>
- Pre-column derivatization of sheep plasma, animal feeds and tissues<sup>8</sup>

## References

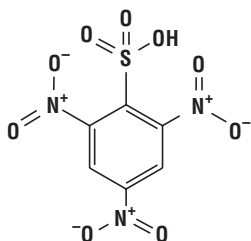
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7. Cooper, J.D.H., et al. (1984). *Anal. Biochem.* **142**, 98-102.
8. Krishnamurti, C.R., et al. (1984). *J. Chromatogr.* **315**, 321-331.
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12. Jones, B.N., et al. (1981). *J. Liq. Chrom.* **4**, 565-586.

## Ordering Information

Product #	Description	Pkg. Size
26015	Fluoraldehyde™ <i>o</i> -Phthalaldehyde Crystals	5 g
20150	Brij®-35 Detergent (30% w/w solution)	950 ml

# TNBSA

An excellent choice for spectrophotometric detection.



TNBSA  
M.W. 293.17

## Highlights:

- Couples with primary amines, sulfhydryls and hydrazides in aqueous solution at pH 8, without undesirable side reactions
- Excellent for solution or solid phase analysis
- Chromogenic ( $\lambda_{\max}$  = 335 nm)

## Ordering Information

Product #	Description	Pkg. Size
28997	TNBSA (2,4,6-Trinitrobenzene sulfonic acid; 5% w/v methanol solution)	100 ml

# Introduction to Ninhydrin Detection of Amino Acids

Ninhydrin-based monitoring systems are among the most widely used methods for quantitatively determining amino acids after they are separated by ion exchange chromatography.

The color reaction between amino-containing compounds and ninhydrin (2,2-dihydroxy-1,3-indandione) is very sensitive. McCaldin has studied all phases of ninhydrin chemistry and proposed a mechanism for the reaction of ninhydrin with amino acids, accounting for the aldehydes, carbon dioxide, ammonia and hydrindantin known to be produced.<sup>1</sup> A yellow colored product (monitored at 440 nm) is formed upon reaction with the secondary amino acids, proline and hydroxyproline.<sup>2</sup> Ninhydrin decarboxylates and deaminates the primary amino acids, forming the purple complex known as Ruhemann's Purple,<sup>3</sup> which absorbs maximally at 570 nm.

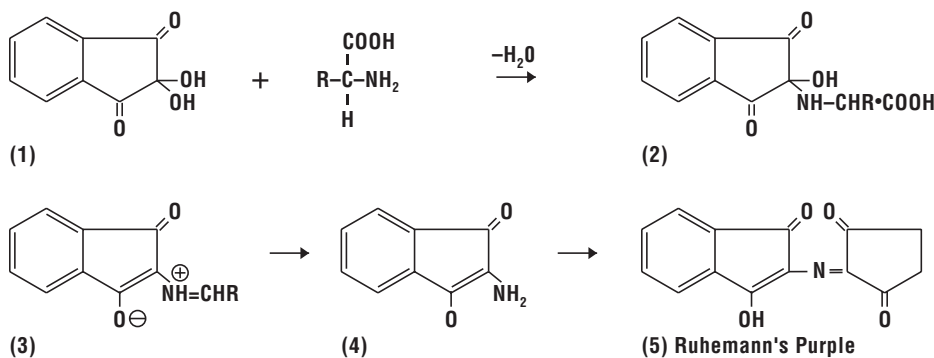
Ninhydrin chemistry was adapted to a fully automatic, two-column amino acid analysis procedure in 1958 by Spackman, Stein and Moore.<sup>4</sup> Moore and Stein defined the requirements for a reducing agent (such as stannous chloride) to achieve reproducible color values for amino acids monitored with ninhydrin.<sup>5</sup> Titanous chloride was reported by James to eliminate precipitates encountered when using stannous chloride.<sup>6-8</sup> Methyl Cellosolve (ethylene glycol monomethyl ether) buffered with 4 M sodium acetate at pH 5.51,<sup>9</sup> and dimethylsulfoxide (DMSO) buffered with 4 M lithium acetate at pH 5.20<sup>10</sup> are the most common solvents used for ninhydrin. DMSO remains stable longer than Methyl

Cellosolve, particularly when kept chilled. These ninhydrin reagent solutions, with increased stability, were also reported by Kirschenbaum.<sup>11</sup> Moore called for substituting Methyl Cellosolve with DMSO, substituting sodium acetate with lithium acetate and including hydrindantin. The role of hydrindantin in determining amino acids was examined by Lamonthé and McCormick.<sup>12</sup>

Sensitivity of the ninhydrin system depends on several factors. Amino acids produce slightly different color yields, and these values may vary from one reagent preparation to the next. Ninhydrin also is sensitive to light, atmospheric oxygen and changes in pH and temperature. When ninhydrin becomes oxidized, its color does not develop well at 570 nm, but absorption at 440 nm remains fairly constant. When the height of the proline peak at 440 nm approaches the height of the glutamic acid peak at 570 nm, for equal amounts of each, reagent degradation is indicated.

## References

1. McCaldin, D.J. (1960). *Chem. Rev.* **60**, 39.
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3. Ruhemann, J. (1911). *J. Chem. Soc. London* **99**, 797.
4. Spackman, D.H., Stein, W.H. and Moore, S. (1958). *Anal. Chem.* **30**, 1190.
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9. Moore, S. and Stein, W.H. (1948). *J. Biol. Chem.* **176**, 367.
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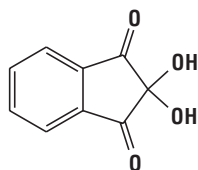


**Reaction Scheme.** The course of the ninhydrin reaction with amino acids is as follows:

1. Ninhydrin (2,2-dihydroxy-1,3-indandione) reacted with amino acid.
2. The intermediate formed as the first reaction product.
3. Intermediate gives rise to dipolar ion by decarboxylation and dehydration.
4. The dipolar ion hydrolyzes, producing the amine.
5. The amine condenses with a second molecule of ninhydrin to give Ruhemann's Purple.

# Ninhydrin

The reagent of choice for detection of amino acids.



**Ninhydrin**  
M.W. 178.14

Since Stein and Moore pioneered amino acid chromatography in 1949,<sup>1</sup> Pierce Ninhydrin has been used in amino acid chromatography advancements. The most recent techniques and sensitive instruments require the superb color response and a low blank that only Pierce Ninhydrin offers.

Pierce Ninhydrin is indefinitely stable and requires no refrigeration or special care. Just keep the bottle tightly sealed, avoiding direct sunlight and ammonia in the laboratory atmosphere.

#### References

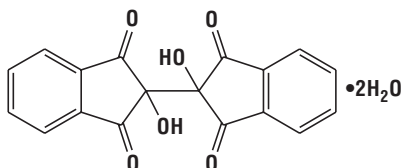
1. Stein, W.H. and Moore, S. (1949). Cold Spring Harbor Symp. *Quant Biol.* **14**, 179.
2. Moore, S. (1968). Amino acid analysis: aqueous dimethyl sulfoxide as solvent for the ninhydrin reaction. *J. Biol. Chem.* **243(23)**, 6281-6283.
3. James, L.B. (1978). Amino acid analysis: ninhydrin reaction with titanous chloride. *J. Chromatogr.* **152**, 298-300.

## Ordering Information

Product #	Description	Pkg. Size
21003	Ninhydrin	500 g

# Hydrindantin

Great for use with Ninhydrin in a DMSO reagent solution.



**Hydrindantin**  
M.W. 358.29

Hydrindantin is a reduced form of Ninhydrin for use with Ninhydrin in a DMSO reagent solution,

or in other systems in which *in situ* generation is not used. The role of hydrindantin in determining amino acids (using Ninhydrin) is discussed by Lamonthé and McCormick.<sup>1</sup> Hydrindantin is supplied in a free-flowing fine powder.

#### References

1. Lamonthé, P.J. and McCormick, P.G. (1973). *Anal. Chem.* **45**, 1906-1911.
2. Moore, S. (1968). Amino acid analysis: aqueous dimethyl sulfoxide as solvent for the ninhydrin reaction. *J. Biol. Chem.* **243(23)**, 6281-6283.

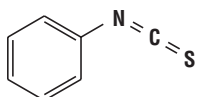
## Ordering Information

Product #	Description	Pkg. Size
24000	Hydrindantin Dihydrate	100 g

# High-Purity Pre-Column Derivatization Reagents

## PITC (Phenylisothiocyanate)

**Ideal for the quantitative pre-column derivatization of amino acids by reverse-phase HPLC.**<sup>1-4</sup>



**PITC**  
Edman's Reagent  
M.W. 135.19

PITC, also known as Edman's Reagent, reacts readily with amino acids in 5-10 minutes at room temperature. The resulting phenylthiocarbonyl derivatives can be separated and quantified in 30 minutes using reverse-phase HPLC. This method produces stable products with all amino acids, including proline.

### To couple Pierce Amino Acid Standard H with PITC.<sup>1</sup>

1. Dry 10  $\mu$ l Amino Acid Standard H in a small test tube. Dissolve dried standard in 100  $\mu$ l coupling buffer (acetonitrile: pyridine: triethylamine: H<sub>2</sub>O, 10:5:2:3).

2. Dry standard solution by rotary evaporation. Dissolve the residual amino acids once more in 100  $\mu$ l of coupling buffer.
3. Add 5  $\mu$ l of PITC.
4. Allow a 5-minute reaction at room temperature.
5. Evaporate sample to dryness by rotary evaporation under high vacuum.
6. Dissolve the resulting PITC-amino acids in 250  $\mu$ l of 0.05 M ammonium acetate, water or water:acetonitrile (7:2).
7. Analyze quantities of 1 to 10  $\mu$ l (100 to 1,000 picomoles of each amino acid) by reverse-phase HPLC.

**NOTE:** Make certain that all HCl is evaporated before derivatization.

#### References

1. Heinrikson, R.L. and Meridith, S.C. (1984). *Anal. Biochem.* **136**, 65-74.
2. Scholze, H. (1985). *J. Chromatogr.* **350**, 453-460.
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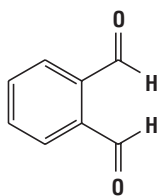
## Ordering Information

Product #	Description	Pkg. Size
✖ 26922	PITC (Phenylisothiocyanate)	10 x 1 ml ampules
20088	Amino Acid Standard H	10 x 1 ml ampules

✖ Additional hazardous handling charge.

# Fluoraldehyde™ Reagent Solution

**Our ready-to-use OPA derivatization reagent for highly sensitive amino acid analysis.**



## Fluoraldehyde™ Reagent Solution (*o*-Phthalaldehyde)

M.W. 134.13

$\lambda_{ex}$  = 340 nm

$\lambda_{em}$  = 455 nm

Pierce ready-to-use Fluoraldehyde™ Reagent Solution is a pre-column amine derivatization reagent that is suitable for amino acid analysis by HPLC.

At room temperature, Fluoraldehyde™ Reagent Solution reacts rapidly with primary amines, and it can be injected into your LC with no further processing.

Fluoraldehyde™ Reagent Solution is ideal for reverse-phase HPLC because the derivatives formed are less polar than free amino acids. All primary amino acids react with Fluoraldehyde™ Reagent, resulting in highly fluorescent isoindole derivatives.

### To couple Pierce Amino Acid Standard H with OPA:

(See page 46 for additional information about Standard H.)

1. Mix 5-10  $\mu$ l of Amino Acid Standard H with 5  $\mu$ l of Fluoraldehyde™ Reagent Solution.
2. After one minute, add 20-100  $\mu$ l of 0.1 M, pH 7.0 sodium acetate to standard solution. Mix.
3. Subject a 20  $\mu$ l aliquot for analysis by reverse-phase HPLC.

**NOTE:** For optimal reproducibility, maintain constant reaction times.

In addition, published papers report that Fluoraldehyde™ Reagent Solution is used in the following applications:

- For pre-column monitoring of amino acid utilization in cell culture<sup>1</sup>
- For hydrolyzate and physiological amino acid derivatization<sup>2</sup>
- Optimal reaction conditions are used to study the on-column amino acid stability<sup>3</sup>
- For hypochlorite oxidation of secondary amino acids<sup>4</sup>
- For pre-column analysis of urinary amino acids<sup>5</sup>
- For use with ethane thiol for improved derivative stability<sup>6,7</sup>
- An oxidation method for proline and secondary amino acids prior to derivatization<sup>8</sup>

### References

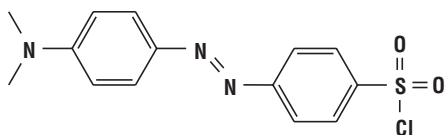
1. Seaver, S.S., *et al.* (Sept./Oct. 1984). *BioTechniques*, 254-260.
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10. Gardner, W.S. and Miller III, W.H. (1980). *Anal. Biochem.* **101**, 61-65.
11. Jones, B.N. and Gilligan, J.P. (1981). *J. Liq. Chromatogr.* **4**, 564-586.
12. Dong, M.W. and DiCesare, J.L. (1983). *LC1*, 222-228.
13. Jones, B.N. and Gilligan, J.P. (1983). *An. Biotech. Lab.*, December, 46-51.
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## Ordering Information

Product #	Description	Pkg. Size
26025	Fluoraldehyde™ Reagent Solution ( <i>o</i> -Phthalaldehyde)	945 ml
26015	Fluoraldehyde™ OPA Crystals	5 g
20088	Amino Acid Standard H	10 x 1 ml ampules

# Dabsyl Chloride

**It's recrystallized twice for twice the quality!**



**Dabsyl Chloride**  
M.W. 323.80

Pierce Dabsyl Chloride is for the pre-column derivatization and detection of amino acids in visible light down to sub-picomolar levels, followed by reverse-phase HPLC.

**Reported applications using Dabsyl Chloride include:**

- Analysis of 10-30 ng of protein hydrolyzates<sup>1,2</sup>
- Analysis of peptides and determination of C-terminal sequence of polypeptides<sup>1</sup>
- Analysis of phospho-amino and amino acid amides<sup>3</sup>
- Analysis of amino acid neurotransmitters in mouse brain<sup>4</sup>
- Optimal reaction conditions<sup>5</sup>

#### References

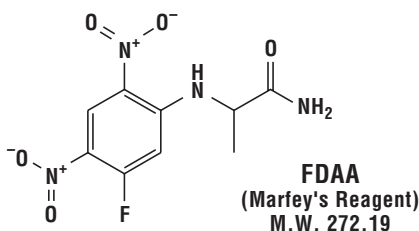
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## Ordering Information

Product #	Description	Pkg. Size
21720	<b>Dabsyl Chloride</b> (4-Dimethylaminoazobenzene-4'-Sulfonyl Chloride)	500 mg

# FDAA, Marfey's Reagent

**Derivatizes optical isomers of amino acids in just 90 minutes.**



Marfey's Reagent, FDAA (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide), offers complete derivatization of amino acid isomers in 90 minutes. Derivatized amino acids then are separated and quantitated by reverse-phase HPLC. The nature of the reagent and the resultant reaction products with D-diastereomers suggest that strong intramolecular hydrogen bonding causes these derivatives to elute much later than their L-diastereomer counterparts. Derivatives have an absorption coefficient of approximately  $3 \times 10^4$ . They can be detected by UV at 340 nm with picomole sensitivity. Complete instructions are included with each order.

**To prepare FDAA Derivatives:**

1. Place 100  $\mu$ l (5  $\mu$ moles) sample in a 1.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Add 200  $\mu$ l of 1% (w/v) solution of FDAA in acetone. Add 40  $\mu$ l of 1.0 M sodium bicarbonate ( $\mu$ moles FDAA:  $\mu$ moles amino acid should be 1.5:1.0.)
3. Heat at 40°C for 1 hour. Remove and cool.
4. Add 20  $\mu$ l 2 M HCl. Allow sample to degas.
5. Analyze. Conditions:  
Spheri-5, RP-18, 10 cm x 4.6 cm  
UV at 340 nm  
A: 0.05 M TEA phosphate pH 3.0  
B: CH<sub>3</sub>CN  
Linear Gradient, 10% B to 40% B in 45 minutes  
Flow: 2.0 ml/min. at 25°C

#### References

1. Marfey, P., *et al.* (1984). *Carlsberg Res. Comm.* **49**, 585-590.
2. Marfey, P. (1984). *Carlsberg Res. Comm.* **49**, 591-596.
3. Szókán, G., *et al.* (1988). Applications of Marfey's Reagent in racemization studies of amino acids and peptides. *J. Chromatogr.* **444**, 115-122.
4. Aberhart, D.J., *et al.* (1985). Separation by high-performance liquid chromatography of (3R)- and (3S)-B-Leucine as diastereomeric derivatives.<sup>1</sup> **151**, 88-91.
5. Martínez del Pozo, *et al.* (1989). Stereospecificity of reactions catalyzed by bacterial D-amino acid transaminase. *J. Biol. Chem.* **264**(30) 17784-17789.

## Ordering Information

Product #	Description	Pkg. Size
48895	<b>FDAA, Marfey's Reagent</b> (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide)	100 mg

# Ultra-Pure Solvents for Amino Acid Analysis

**Ideal for HPLC and spectrophotometric applications.**

Pierce high-quality solvents are distilled in glass and filtered through 0.2 micron Teflon® TFE membranes. Stringent quality control specifications assure that all solvents are suitable for both HPLC and spectrophotometric applications.

All Pierce HPLC/spectro grade solvents are packed in solvent-rinsed, amber glass bottles with Teflon® TFE-lined screw caps seal bottles for ultimate protection.

Label information includes formula, molecular weight, safety data, flashpoint, first aid procedure, UV absorbance data, GC assay, evaporation residue and water content.

**Table 1.**

Product	UV Cutoff	Optical Absorbance	Refraction Index (@ 25°C)
Acetonitrile	190 nm	0.02 at 230 nm	1.342
Water	190 nm	0.005 at 250 nm	1.332

## Ordering Information

Product #	Description	Pkg. Size
× 51101	Acetonitrile	1 L
51140	Water	1 L

× Additional hazardous handling charge.



# HPLC

Ionate™ Ion Pair Reagents ....p.58-59

HPLC/Spectro Grade Solvents ...p.60

Peptide Retention Standard .....p.61

Derivatization/  
Visualization Reagents .....p.62-64

Detection Reagents  
Selection Guide.....p.65

References.....p.66

**PIERCE**

# Ionate™ Ion Pair Reagents

**High-purity reagents with the selectivity needed for good separations.**

In the past, reverse-phase HPLC analysis of highly charged acidic and basic compounds was frustrating and resulted in poor resolution. Important biomolecules such as amino acids, peptides, organic acids, polyamines and catecholamines had to be separated by ion exchange or by suppression techniques.

Ionate™ Ion Pair Reagents enable you to quickly and efficiently analyze charged compounds using reverse-phase techniques. The Ion Pair Reagents are simply dissolved in the HPLC solvent system, resulting in the formation of stable chromatographic complexes that can be separated using reverse-phase columns. By using the correct Ion Pair Reagents, you achieve:

- Increased or decreased retention, permitting controlled selectivity
- Resolution of complex ionic mixtures without using ion exchange columns
- Improved peak symmetry

## Reverse-phase ion pair chromatography theories

Two principal theories have been proposed to explain reverse-phase ion pair chromatography. In the first theory, small polar ion pair reagents react with the ionized solute, forming neutral ion pairs. The second theorizes that an active ion exchange surface is produced in which long chain, nonpolar anions and cations are absorbed by the hydrophobic stationary phase.

To optimize chromatographic separations in ion pair elution systems, high-purity reagents of exceptional optical transparency are needed. Ionate™ Ion Pair Reagents are specially purified for ion pair chromatography and provide the selectivity needed for good separations.

### References

1. Bennett, H.P.S., *et al.* (1981). *Biochemistry*. **20**, 4530.
2. Starratt, A.N. and Stevens, M.E. (1980). *J. Chromatogr.* **194**, 421.
3. Burgess, A.W., *et al.* (1982). *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5753.
4. Hearn, M.T.W. and Grego, B. (1983). *J. Chromatogr.* **255**, 125.
5. Shonshofer, M. and Fenner, A. (1981). *J. Chromatogr.* **224**, 472.
6. Fischli, W., *et al.* (1982). *Proc. Natl. Acad. Sci. U.S.A.* **79**, 5435.
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8. Hearn, M.T.W., *et al.* (1978). *J. Chromatogr.* **157**, 337.
9. Hearn, M.T.W. and Grego, B. (1983). *J. Chromatogr.* **255**, 125.
10. Hearn, M.T.W. and Grego, B. (1983). *J. Chromatogr.* **266**, 75.
11. Rivier, J. (1978). *J. Liq. Chrom.* **1**, 343.

## Trifluoroacetic Acid (TFA)

**All trifluoroacetic acids are not equal – demand what is crucial for your application.**

### Highlights:

- Purity – exhibits superior purity ( $\geq 99.5\%$ ) and exceptional clarity, allowing for sensitive, non-destructive peptide detection at low UV wavelengths in reverse-phase HPLC protein and peptide separation systems.<sup>1</sup>
- Versatility – performs incomparably in protein sequencing applications<sup>2,3</sup> and solid-phase peptide synthesis<sup>4</sup> and as a protein/peptide solubilizing agent.<sup>2,3</sup>
- High-performance packaging – TFA is an extremely corrosive organic solvent and vapors corrode conventional bottle caps. Damaged packaging endangers TFA purity and it sabotages one's work, wasting valuable time. Pierce packages TFA in amber glass with protective Teflon® TFE-lined fluorocarbon caps or ampuled under nitrogen.

- Economical convenience – available in a variety of package formats and sizes. Save money by choosing the package that works best for your specific applications. For example, the 1 ml ampules provide a simple way to prepare liter quantities of 0.1% TFA for stationary and mobile phases in reverse-phase chromatography.

### References

1. Chicic, R.M. and Regnier, F.E. (1990). *Methods Enzymol.* **182**, 392-421.
2. Smith, B.J. (1997). *Protein Sequencing Protocols*. Humana Press.
3. Allen, G. (1989). *Sequencing of Proteins and Peptides*, Second Revised Edition. Elsevier.
4. Stuart, J.M. and Young, J.D. (1984). *Solid Phase Peptide Synthesis*, Second Edition. Published by Pierce Chemical Company.

# Heptafluorobutyric Acid

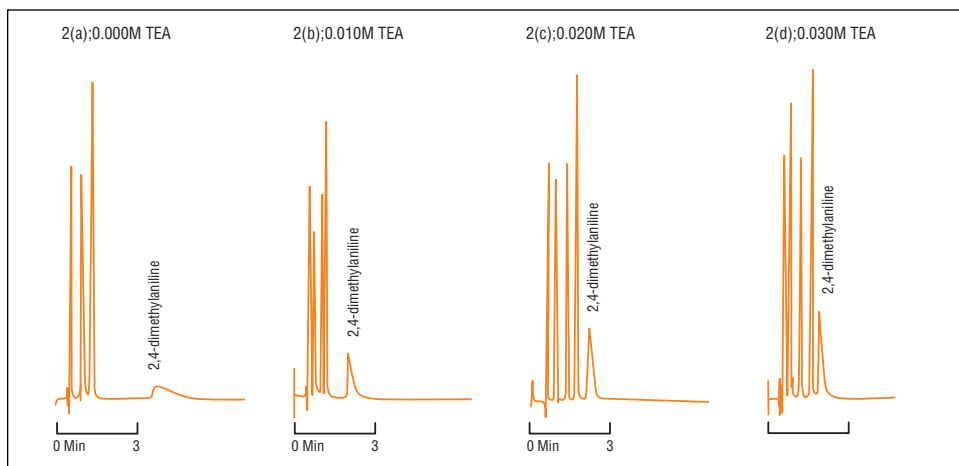
**An ion pair reagent for the reverse-phase HPLC separation of proteins and peptides.**

## Highlights:

- Clear, colorless liquid
- Typical purity is 99.7% by GC; less than 0.1% water
- Sequencing reagent for classical and automated Edman degradation of peptides and proteins
- Density: 1.645; B.P.: 120°C

## References

1. Hearn, M.T.W. and Hancock, W.S. (1979). *Trends Biochem. Sci.* **4**, N58-N62.
2. Bennett, H.P.J., et al. (1980). *J. Liquid Chromatogr.* **3**, 1353-1366.
3. Bennett, H.P., et al. (1981). *Biochemistry* **20**, 4530-4538.



**Figure 1A-1D.** Effect of TEA concentration on a mixture of basic antihistamines and 2,4-dimethylaniline\* 15 cm x 4.6 mm Zorbax® C<sub>8</sub>.

Conditions: a) 40% methanol, 0.060 M HSA sodium salt, 0.045 M citric acid; b) 0.150 M citric acid, 0.060 M TEA, pH 7.5 with NaOH; c) 0.150 M citric acid, pH 7.5 with NaOH; isocratic with TEA concentrations modified by varying b/c ratio, 3 min./ml, 50°C, 254 nm.

## Ionate™ Ion Pair Reagents

### Ordering Information

#### Perfluorinated Alkanoic Acids

Product #	Description	Pkg. Size
✘ 53104	Heptafluorobutyric Acid	10 x 1 ml
✘ 28901	Trifluoroacetic Acid, Sequanal Grade	500 ml
✘ 28902	Trifluoroacetic Acid, Sequanal Grade	10 x 1 g
✘ 28903	Trifluoroacetic Acid, Sequanal Grade	100 g
✘ 28904	Trifluoroacetic Acid, Sequanal Grade	10 x 1 ml ampules

#### Tertiary Alkylamine

Product #	Description	Pkg. Size
✘ 53101	Triethylamine	25 g

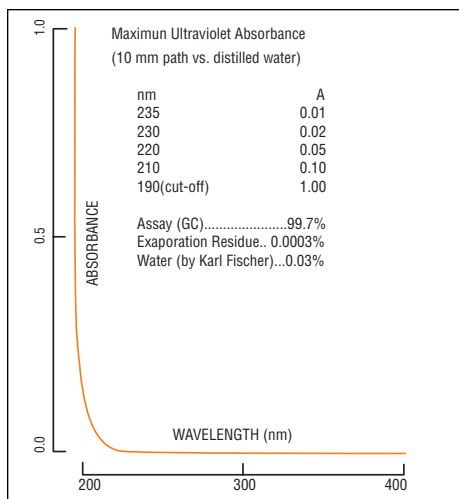
#### Meptfluorobutyric Acid

Product #	Description	Pkg. Size
✘ 25003	Heptafluorobutyric Acid*, Sequanal Grade	100 ml
✘ 53104	Ionate™ Heptafluorobutyric Acid, HPLC Grade	10 x 1 ml

✘ Additional hazardous handling charge.  
\* Also referred to as Sequentor Reagent 3.

# HPLC/Spectro Grade Solvents

**Ultra-pure solvents for HPLC and spectrophotometric applications.**



**Figure 1.** UV spectrum of acetonitrile; Path Length: 10 mm; Reference: H<sub>2</sub>O; Range: 190-400 nm.

Pierce HPLC/Spectro Grade Solvents are ultra-pure, distilled in glass, filtered through 0.2 micron Teflon® TFE membranes and packed in solvent-rinsed, amber glass bottles. Teflon® TFE-lined screw caps seal the bottles for ultimate protection.

Label information includes formula, molecular weight, safety data, flashpoint, first aid procedure, UV absorbance data, GC assay, evaporation residue and water content.

**Table 1.**

Solvent	Unit	UV Cutoff	Optical Absorbance	Refractive Index (@ 25°C)
Acetonitrile	1 L	190 nm	0.02 at 230 nm	1.342
Water	1 L	190 nm	0.005 at 250 nm	1.332

## Ordering Information

Product #	Description	Pkg. Size
✘ 51101	Acetonitrile	1 L
51140	Water	1 L

✘ Additional hazardous handling charge.

# Peptide Retention Standard for Reverse-Phase HPLC

## Increases the efficiency of peptide elution profile predictions.

A simple, quantitative method for predicting peptide retention times was developed by Guo, *et. al.*<sup>1-3</sup> Retention times are predicted by totaling the values that represent the contribution in minutes of each amino acid residue and the peptide terminal groups.

Retention time is dependent upon the molecular weight of the peptide. The effect on retention is relatively unimportant with a small peptide, but it increases with the size of the molecule. The accuracy of predicting peptide retention time significantly decreases beyond 20 residues.

To ensure accuracy, a peptide standard is used to correct for instrument variation, column aging, n-alkyl chain length variation and ligand density.

By using Pierce Peptide Retention Standard, you can:

- Determine the relative order of peptide elution of a complex mixture
- Increase the efficiency of predicting peptide elution profiles
- Save time in peptide purification

- Simplify identification of specific peptides in a complex mixture
- Predict the HPLC retention time for peptides of known amino acid composition on reverse-phase HPLC columns
- Monitor column performance – efficiency, selectivity and resolution during column aging
- Compare reverse-phase columns from different manufacturers
- Evaluate reverse-phase supports of varying n-alkyl chain lengths and ligand densities

### References

1. Guo, D., *et al.* (1985). *Proceedings of the Ninth American Peptide Symposium*, Published by Pierce, Rockford, Illinois, page 23.
2. Guo, D., *et al.* (1986). *J. Chromatogr.* **359**, 499-517.
3. Guo, D., *et al.* (1986). *J. Chromatogr.* **359**, 519-532.
4. Mant, C.T. and Hodges, R.S. (1986). *L.C. Magazine Liq. Chrom. and HPLC* **4(3)**, 250.
5. Guo, D., *et al.* (1987). *J. Chromatogr.* **386**, 205-222.

## Ordering Information

Product #	Description	Pkg. Size
31700	<b>Peptide Retention Standard, S1-S5</b> Contains: 5 C-terminal amide decapeptides, 4 of which are N <sup>α</sup> -acetylated with the sequence variation as follows: AC-Arg-Gly-X-X-Gly-Leu-Gly-Leu-Gly-Lys-Amide; Gly <sup>2</sup> -Gly <sup>4</sup> -Ala <sup>2</sup> -Gly <sup>4</sup> , Val <sup>2</sup> -Gly <sup>4</sup> and Val <sup>2</sup> -Val <sup>4</sup> . The fifth peptide, Ala <sup>2</sup> -Gly <sup>4</sup> , contains a free N $\alpha$ -amino group. This mixture will provide 100-200 injections at 0.1 AUFS at 210 nm.	1 vial

# Introduction to Pierce Derivatization Reagents for HPLC

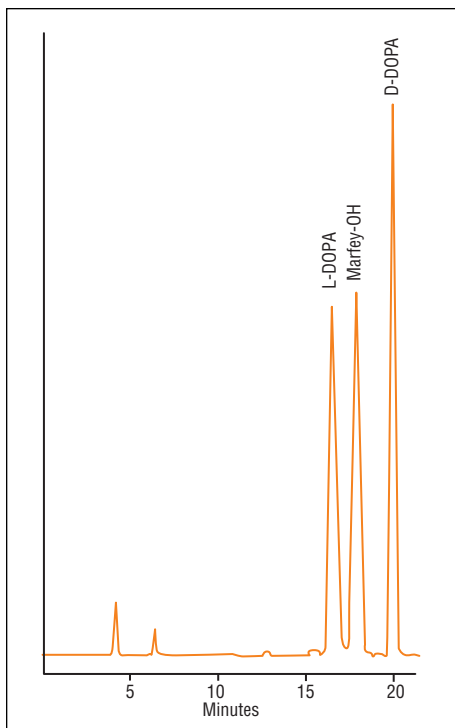
**Designed to provide selectivity and improve sensitivity.**

The lack of a universal HPLC detector that provides high sensitivity (as well as some degree of selectivity) established the need for suitable derivatization procedures. Derivatization is the chemical modification of an existing compound, producing a new compound that has properties more suitable for a specific analytical procedure. It is an analytical tool that can be used to provide both selectivity and improved sensitivity.

There are several requirements for derivatization protocol:

1. At least one acidic, polar functional group must be available for reaction on the parent compound.
2. A single derivative should be formed per parent compound.
3. The reaction should be reproducible under the given time and reaction conditions.
4. The reaction should proceed quickly and easily under mild conditions.
5. The reaction byproducts (if any) should not interfere with the chromatography, or with detection of the sample.

Pre- and post-chromatographic techniques are both used in HPLC derivatization. In addition, off-line and on-line reactions have been employed with both techniques. Pre-chromatographic (or pre-column techniques) offer more than greater selectivity and sensitivity in detection. Pre-column techniques can be used to enhance stability, improve resolution, improve peak symmetry and increase or decrease retention of solutes. FDAA (Marfey's Reagent) allows separation and quantification of optical isomers of amino acids (Figure 1). Post-chromatographic (or post-column) techniques are used primarily to provide selectivity and improve sensitivity.



**Figure 1.** Separation of D- and L-DOPA on Spheri-5™ Sorbent, RP-18. 10 cm x 4.6 mm  
Conditions: A) 0.05 M triethylamine phosphate, pH 3.0; B) acetonitrile. Linear gradient: 10 to 40% B in 45 minutes, 2.0 ml/minute, 25°C, 340 nm.

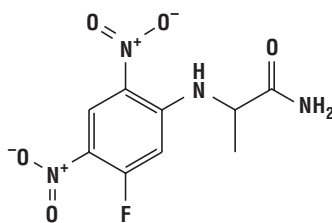
Pierce offers a variety of HPLC detection reagents for pre- and post-chromatographic techniques. All compounds and formulations are purified for chromatography, minimizing artifact formation.

# FDAA, Marfey's Reagent

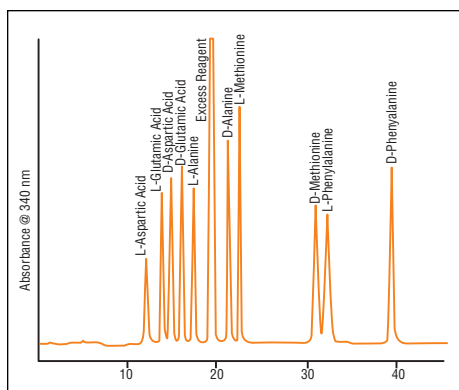
**Makes it quick and easy for you to separate and quantitate optical isomers of amino acids by reverse-phase HPLC.**

Optical isomers of amino acids can be simply and conveniently derivatized with Marfey's Reagent [FDAA (1-fluoro-2,4-dinitro-phenyl-5-L-alanine amide)] – and preparation is complete in just 90 minutes.

With Marfey's Reagent, the amino acid derivatives can easily be separated and quantitated by reverse-phase HPLC. Derivatives have an absorption coefficient of  $\sim 3 \times 10^4$  and can be detected by UV at 340 nm with picomole sensitivity.



**FDAA**  
**(Marfey's Reagent)**  
M.W. 272.19



**Figure 1.** Separation of D- and L-amino acids on Spheri-5™ Sorbent, RP-18. 10 cm x 4.6 mm. Conditions: A) 0.05 M triethylamine phosphate, pH 3.0; B) acetonitrile. Linear gradient: 10 to 40% B in 45 minutes, 2.0 ml/minute, 25°C, 340 nm.

## PROTOCOL

### Preparation of FDAA Derivatives

1. Place 100  $\mu$ l (5  $\mu$ mol) sample in a 1.0 ml Reacti-Vial™ Small Reaction Vial (see page 68).
2. Add 200  $\mu$ l of a 1% (w/v) solution of FDAA in acetone. Add 40  $\mu$ l of 1.0 M sodium bicarbonate. Total FDAA:  $\mu$ mol amino acid should be 1.5:1.0.
3. Heat at 40°C for 1 hour. Remove and cool.
4. Add 20  $\mu$ l 2 M HCl. Allow sample to degas.
5. Analyze. Conditions:  
Spheri-5™ Sorbent, RP-18, 10 cm x 4.6 cm  
UV at 340 nm  
A: 0.05 M TEA phosphate, pH 3.0  
B: CH<sub>3</sub>CN  
Linear gradient: 10% B to 40% in 45 minutes  
Flow: 2.0 ml/minute at 25°C

### Reference

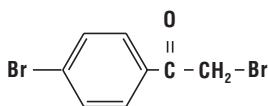
1. Marfey, P. (1984). *Carlsberg Res. Comm.* **49**, 591-596.

## Ordering Information

Product #	Description	Pkg. Size
48895	<b>FDAA, Marfey's Reagent</b> (1-fluoro-2,4-dinitrophenyl-5-L-alanine-amide)	100 mg

# *p*-Bromophenacyl-8™ Reagent

Procedure gives quantitative yields with few or no side reactions.



***p*-Bromophenacyl-8™**  
M.W. 277.94

Durst, *et al.* have described a novel preparation of various phenacyl esters and their use as UV visualizing agents in the 1-10 ng range. This procedure gives quantitative yields with few or no side reactions. Phenacyl esters have been used to separate many saturated and unsaturated fatty acids,<sup>2,3</sup> including prostaglandins.<sup>4</sup>

Phenacyl esters have some significant advantages over previously reported methods, including:

- Pre-mixing of phenacylbromide and crown ether is not necessary
- Derivatization is both rapid and quantitative, with yields of more than 95% in 15-20 minutes at 80°C
- Excess reactants do not interfere
- Large excess of alkylating reagent is not necessary
- Small amounts of water or alcohol do not interfere
- If isolation is desired, products usually are crystalline

## PROTOCOL

### Preparation of Phenacyl Esters

***p*-Bromophenacyl-8™ Reagent** (0.1 μmol/ml *p*-Bromophenacylbromide, 0.005 μmol/ml crown ether in acetonitrile)

1. Dissolve ~10 mg acid in MeOH in a 5.0 ml Reacti-Vial™ Small Reaction Vial (page 68) fitted with Reacti-Vial™ Magnetic Stirrer (page 82). Neutralize to the phenolphthalein endpoint with KOH/MeOH.\*
2. Evaporate the MeOH with N<sub>2</sub>.
3. Add 1.0 ml Phenacyl-8™ Reagent and 2.0 ml dry CH<sub>3</sub>CN.
4. Heat at 80°C with stirring for 30 minutes.
5. Remove and cool.
6. Analyze. Conditions:  
Spheri-5™, RP-18  
UV at 250 nm  
A: CH<sub>3</sub>CN  
B: deionized H<sub>2</sub>O  
Linear gradient: 80% A to 100% A  
Flow: 2.0 ml/minute

\* If the formation of potassium salts is undesirable, neutralize by adding KHCO<sub>3</sub> at five times the total acid instead of using KOH.

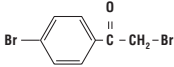
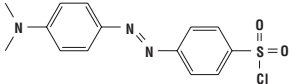
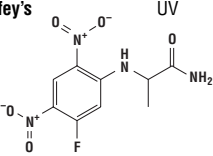
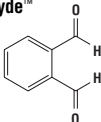
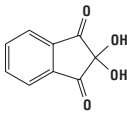
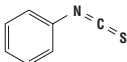
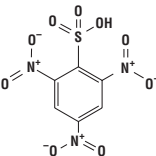
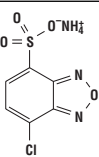
### References

1. Durst, H.D., *et al.* (1975). *Anal. Chem.* **47**, 1797.
2. Borch, R.F., *et al.* (1975). *Anal. Chem.* **47**, 2437.
3. Grushka, E., *et al.* (1975). *J. Chromatogr.* **112**, 673.
4. Fitzpatrick, F.A. (1976). *Anal. Chem.* **48**, 499.
5. Nagels, L., *et al.* (1980). *J. Chromatogr.* **190**, 411.
6. Ahmed, M.S., *et al.* (1980). *J. Chromatogr.* **192**, 387.
7. Pierce Technical Bulletin: Preparation of phenacyl and *p*-Bromophenacyl derivatives for HPLC.
8. Patience, R.L. (1982). *J. Chromatogr.* **249**, 183-186.

## Ordering Information

Product #	Description	Pkg. Size
48891	<b><i>p</i>-Bromophenacyl-8™ Reagent</b> 0.1 mmol/ml <i>p</i> -Bromophenacylbromide, 0.005 mmol/ml crown ether in acetonitrile	10 ml Hypo-Vial™ Sample Storage Vial

# Detection Reagents for Specific Functional Groups

Functional Group	Description	Detection*	Page	Comments
<b>Carboxylic Acid</b> $\begin{array}{c} \text{O} \\    \\ \text{R}-\text{C}-\text{OH} \end{array}$	<b><i>p</i>-Bromophenacyl-8<sup>TM</sup></b> 	UV	64	Formulation of 1.0 mmol/ml <i>p</i> -bromophenacyl bromide and 0.005 mmol/ml crown ether in acetonitrile; pre-column; nanomole detection levels: $\lambda_{\text{max}} = 260 \text{ nm}^{17}$
<b>Primary Amine</b> $\begin{array}{c} \text{R}-\text{N}-\text{H} \\   \\ \text{H} \end{array}$	<b>Dabsyl Chloride</b> 	Vis	55	4-N, N-dimethylaminoazobenzene-4'-sulfonyl chloride (dabsyl chloride); pre-column; nanomole detection levels: $\lambda_{\text{max}} = 436 \text{ nm}^{8,14}$
	<b>FDAA, Marfey's</b> 	UV	55	1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (FDAA); pre-column; nanomole detection levels: $\lambda_{\text{max}} = 340 \text{ nm}$ . For chiral separations of amino acids. <sup>15, 28-29</sup>
	<b>Fluoraldehyde<sup>TM</sup></b> 	EC, F	54	Formulation of 0.8 mg OPA/ml in 1 M potassium borate buffer, pH 10.4 containing Brij <sup>®</sup> -35 and $\beta$ -mercaptoethanol; pre- or post-column; picomole detection levels with EC and F; GC + 0.5 1.0 V; $\lambda_{\text{exc}} = 360 \text{ nm}$ , $\lambda_{\text{em}} = 455 \text{ nm}^{16,21}$
	<b>Ninhydrin</b> 	Vis	52	Post-column; nanomole detection levels: $\lambda_{\text{max}} = 570 \text{ nm}^{22}$
	<b>PITC</b> 	UV	53	Phenylisothiocyanate (PITC); pre-column; picomole detection levels: $\lambda_{\text{max}} = 254 \text{ nm}^{23,24}$
	<b>TNBSA</b>  <b>TNBSA</b> M.W. 293.17	EC, UV	50	2,4,6-Trinitrobenzene-sulfonic acid (TNBSA); pre- or post-column; nanomole detection levels with EC and UV, GC - 0.85V; $\lambda_{\text{max}} = 250 \text{ nm}^{25,26}$
<b>Secondary Amine</b> $\text{R}-\text{NH}-\text{R}'$	<b>Ninhydrin</b> (see structure above)	Vis	52	Post-column; nanomole detection levels: $\lambda_{\text{max}} = 440 \text{ nm}^{22}$
	<b>PITC</b> (see structure above)	UV	53	Phenylisothiocyanate (PITC); pre-column; picomole detection levels: $\lambda_{\text{max}} = 254 \text{ nm}^{23,24}$
<b>Thiol</b> $\text{R}-\text{SH}$	<b>SBF-Chloride</b> 	F	66	Ammonium-4-chloro-7-sulfobenzofurazan (SBF-Cl); pre-column; picomole detection levels: $\lambda_{\text{exc}} = 390 \text{ nm}$ , $\lambda_{\text{em}} = 510 \text{ nm}^{27}$

\*EC = electrochemical; F = fluorescence; UV = ultraviolet; Vis = visible.

# Detection Reagents

Additional Pierce Detection Reagents – Here's how to order:

## Ordering Information

Product #	Description	Pkg. Size
26035	<b>SBF-Chloride</b> (Ammonium 4-Chloro-7-sulfobenzofurazan)	100 mg

### References

1. Durst, H.D., *et al.* (1975). *Anal. Chem.* **47**, 1797.
2. Borch, R.F., *et al.* (1975). *Anal. Chem.* **47**, 2437.
3. Grushka, E. *et al.* (1975). *J. Chromatogr.* **112**, 673.
4. Fitzpatrick, F.A. (1976). *Anal. Chem.* **48**, 499.
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13. Lin J.K., *et al.* (1980). *Anal. Chem.* **52**, 630-635.
14. Chang, J.Y., *et al.* (1981). *FEBS Letters* **132**, 117-120.
15. Marfey, P. (1984). *Carlsberg Res. Comm.* **49**, 591-596.
16. Jones, B.N. and Gilligan, J.P. (1983). *Amer. Biotech. Lab.* **12**, 46-51.
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23. Heinrickson, R.L. and Meredith, S.C. (1984). *Anal. Biochem.* **137**, 65-74.
24. Scholze, H. (1985). *J. Chromatogr.* **350**, 453-460.
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26. Caudill, W.L., *et al.* (1982). *Bioanalytical System's Current Separations* **4(4)**, 59.
27. Andrews, J.L., *et al.* (1982). *Arch. Biochem. Biophys.* **214**, 386-396.
28. Aberhart, D.J., *et al.* (1985). *Anal. Biochem.* **151**, 88-91.
29. Szokan, G., *et al.* (1988). *J. Chromatogr.* **444**, 115-122.

A photograph of laboratory glassware, including a large Erlenmeyer flask in the foreground and a graduated cylinder in the background. The glassware contains liquids of various colors: red, purple, yellow, and blue. The background is blurred, showing more laboratory equipment and colorful lighting.

# Sample Handling

Vials and Closures .....p.68-74

Cleaning Agents .....p.75-76

Heating/Stirring/  
Evaporation .....p.77-82

**PIERCE**

# Reacti-Vial™ Small Reaction Vials

**Make small-sample handling easy and convenient.**



## Ideal for:

- Residue isolation
- Derivative preparation
- Maximum sample retrieval
- Moisture protection
- Sample storage

Reacti-Vial™ Small Reaction Vials have an internal cone designed to make small-sample collection and handling easy and convenient. The cone feature is particularly useful for removing small quantities of sample with a syringe, even into the microliter range. The extra thick glass wall magnifies the sample, making these units ideal for observing chemical reactions. Reacti-Vial™ Small Reaction Vials can be used for derivatization, isolation and purification. You can also use Reacti-Vial™ Small Reaction Vials for precipitations, centrifugations and solvent separations.

Our amber Reacti-Vial™ Small Reaction Vials are manufactured from amber glass, and are amber throughout. These amber vials assure that your light-sensitive compounds are well protected. All Pierce Reacti-Vial™ Small Reaction Vials are supplied complete with Open-Top Screw Caps and Teflon®/Rubber Laminated Discs (other discs can be ordered separately, see page 71).

## Reacti-Vial™ Small Reaction Vials

Clear Pkg. of 12		Amber Pkg. of 12		Dimensions (Diam. x Height) (mm ± 1mm)	Outside Diameter (mm)
Number	Size	Number	Size		
13100	100 µl			12 x 32	8
13220	0.3 ml			13 x 32	11
13221		13097	1.0 ml	13 x 45	11
13222			3.0 ml	20 x 47	18
13223		13099	5.0 ml	20 x 60	18
13225			10.0 ml	25 x 69	22

## Optional Accessories

Vial Size	Tuf-Bond™ Teflon®/ Silicone Discs	Tuf-Bond™/ Rubber Laminated Discs	Open-Top Screw Caps	Mininert® Valves	Reacti-Vial™ Magnetic Stirrers
	Number	Number	Number	Number	Number
100 ml	12708		13208		
0.3 ml	12712	12412	13215		16010
1.0 ml	12712	12412	13215		16010
3.0 ml	12718	12418	13218	10135	16000
5.0 ml	12718	12418	13218	10135	16000
100 ml	12722	12422	13219	10130	16000

# Vari-Clean™ Pre-Cleaned Water Sampling Vials

**We pre-clean our vials to save you time and hassle.**

## 40 ml EPA\* Water Sample Kits

For sampling to analyze priority pollutants (including trihalomethanes), Pierce pre-cleaned water sampling vials are a great time-saver. Ready for immediate use, Vari-Clean™ Pre-Cleaned Water Sampling Vials eliminate the time, inconvenience and expense of cleaning and drying your sampling vials. Each Vari-Clean™ 40 ml Vial package includes 72 pre-cleaned, fully

assembled 40 ml Screw Cap Septum Vials. (Product #13075) and Tuf-Bond™ Teflon®/Silicone Discs (Product #12722) in convenient-to-use shrink-wrapped divider trays.

*\*Pre-cleaned for discrete water sampling according to EPS 40 CFR 136 "Guidelines for Establishing Test Procedures for the Analysis of Pollutants" and EPA 40 CFR 141 "National Interim Primary Drinking Water Regulations; Control of Trihalomethanes in Drinking Water."*

## Ordering Information

Product #	Description	Pkg. Size
13510	Vari-Clean™ 40 ml Pre-Cleaned Water Sampling Vials	Pkg. of 72

# Vari-Clean™ Pre-Cleaned Vials

**Versatile vials for your sampling, analytical and general laboratory needs.**



## Advantages

- Allow instant syringe access to reagents and standards through the open top screw caps
- Long-term sample storage of biological media and volatile solutions
- Ideal for sample collection and testing

Vari-Clean™ Sample Storage Vials provide a meticulously cleaned, ready-to-use vial system. Because they contain no residue from the manufacturing process, you can eliminate the

inconsistency, inconvenience and expense of cleaning and drying sampling and storage vials.

Tuf-Bond™ Cap Liners compress to tightly seal your sample. Tuf-Bond™ Discs are autoclavable and resealable. The silicone body (90 mils thick) and the Teflon® Face (10 mils) are structurally not cemented like most cap liners. So, no cement can be leached or baked out after needle penetration. The Teflon® Face provides an inert barrier between your sample and the screw cap.

Vari-Clean™ Vials are washed, dried and shrink-wrapped for contaminant-free shipping. Each package consists of 72 fully assembled screw cap vials and Tuf-Bond™ Teflon®/Silicone Discs in convenient-to-use, shrink-wrapped divider trays.

## Ordering Information

Product #	Description	Pkg. Size
13504	Vari-Clean™ Pre-Cleaned Vials	3.5 ml, clear, pkg. of 72
13510	Vari-Clean™ Pre-Cleaned Vials	40.0 ml, clear, pkg. of 72

# Hypo-Vial™ Chemical Storage System

Pierce's convenient system for storage and handling of chemical materials includes high-quality amber or clear borosilicate glass vials in a variety of sizes, a selection of discs and septa that

are compatible with a wide range of materials (page 71), and easy-to-use tools for applying and removing seals.

## Hypo-Vial™ Sample Storage System

**Made of high-quality borosilicate glass for heating or autoclaving.**



*Hypo-Vial™ Sample Storage Vials*



*DeKapitator™ Seal Remover*

### Highlights:

- 20 mm (except 1 mm vial, which is 13 mm)
- Instant syringe access to completely sealed reagents and standards
- Light-blocking amber glass available for complete protection of light-sensitive compounds

## Hypo-Vial™ Sample Storage Vials

Product #	Description	Pkg. Size
12903	Hypo-Vial™ Sample Storage Vials	6.0 ml, clear, Pkg. of 72
12944	Hypo-Vial™ Sample Storage Vials	30.0 ml, clear, Pkg. of 72
12969	Hypo-Vial™ Sample Storage Vials	50.0 ml, clear, Pkg. of 72
12995	Hypo-Vial™ Sample Storage Vials	125.0 ml, clear, Pkg. of 84

## DeKapitator™ Seal Removers

Product #	Description
13210	Removes 20 mm seals from 6-125 ml Hypo-Vial™ Sample Storage Vials
13200	Removes 13 mm seals from 1 ml Hypo-Vial™ Sample Storage Vials

# Discs and Septa

**A variety of choices to fit your exact needs.**



Teflon®/Rubber  
Laminated Discs



Tuf-Bond™ Teflon®/  
Silicone Discs



Butyl Septa



Hycar Septa



Silicone Septa

## For use with 1 ml Hypo-Vial™ Sample Storage Vials

Product #	Description	Pkg. Size
12413	Teflon® Rubber Laminated Discs	Pkg. of 72

## For use with 6, 10, 15, 30, 50 or 125 ml Hypo-Vial™ Sample Storage Vials

Product #	Description	Pkg. Size
12720	Tuf-Bond™ Teflon®/Silicone Discs	Pkg. of 72
13050	Gray Butyl Septa	Pkg. of 72
13230	Gray Hycar Septa	Pkg. of 72
13237	Silicone Septa	Pkg. of 72

*Testing against sample material is recommended before extended use. Refer to our septa compatibility guide for assistance in choosing the best septa or disc for your application (see page 74).*

# Screw Cap Septum Vials

**Autoclavable, borosilicate glass available in clear or amber.**



For economy, convenience and versatility in a vial and closure system, Pierce Screw Cap Septum Vials are your best choice. A wide assortment of special closures and accessories make this system perfect for:

- Storage of reagents and standards under complete seal with instant syringe access
- Small derivatization reactions

- Sample collection – the 40 ml clear or amber vial with a Tuf-Bond™ Disc is suitable for discrete water sampling under EPA 40 CFR Parts 136 and 141
- Automated GCs and LCs – the 1.5 ml clear and amber vials fit autosamplers using standard 12 x 32 mm vials
- Heavy-duty, flip-top divider box provides easy access to vials; caps and septa and offers a convenient sample storage center

Pierce Screw Cap Septum Vials are supplied complete with Open-Top Screw Caps. See page 74 for septa available from Pierce.

## Screw Cap Septum Vials

Clear Pkg. of 72	Amber Pkg. of 72	Size	Dimensions (Diam. x Height) (mm ± 1 mm)	Outside Diameter (mm)
	<b>13080</b>	1.5 ml	12 x 32	8
<b>13019</b>		3.5 ml	15 x 45	12
<b>13028</b>		7.0 ml	17 x 60	13
<b>13043</b>		14.0 ml	21 x 70	16
<b>13074</b>		25.0 ml	27 x 70	22
<b>13075</b>		40.0 ml	27 x 95	22

## Optional Accessories

Vial Size	Tuf-Bond™ Teflon®/ Silicone Discs Pkg. of 72	Teflon®/Rubber Laminated Discs Pkg. of 72	Mininert® Valves Pkg. of 12	Open-Top Screw Caps Pkg. of 72
	Number	Number	Number	Number
1.5 ml	<b>12708</b>			<b>13208</b>
3.5 ml	<b>12712</b>	<b>12412</b>		<b>13215</b>
7.0 ml	<b>12713</b>	<b>12413</b>		<b>13216</b>
14.0 ml	<b>12716</b>			<b>13217</b>
25.0 ml	<b>12722</b>	<b>12422</b>	<b>10130</b>	<b>13219</b>
40.0 ml	<b>12722</b>	<b>12422</b>	<b>10130</b>	<b>13219</b>

# Tuf-Bond™ Discs

**Unique discs that combine the inertness of a Teflon® Coating with the resealability of silicone.**



Autoclavable Tuf-Bond™ Discs are specifically designed to combine the resealability of silicone with the inertness of a Teflon® Coating. Many sizes are available to fit our Hypo-Vial™, Reacti-Vial™ Screw Cap and

Tuf-Tainer™ Vials. Additional sizes are available for use as cap liners on 4, 8 and 16 oz. bottles.

**Here are some of the features that make Tuf-Bond™ Discs the ideal closure for many Pierce vials and bottles:**

- Structurally bonded (not cemented) Teflon® Coating to silicone. No cement to be leached or baked out of your sample after needle penetration
- Reseals instantly puncture after puncture
- Compresses, giving it a “lock-washer” effect in maintaining a tight seal, and forces the Teflon® Coating to conform to the sealing surface
- No bent needles from a septum that is too hard; standard syringe and CC needles penetrate the entire disc with ease
- Protection of Teflon® Coating: The Teflon® layer is a full 10 mils thick

## Tuf-Bond™ Discs (pkg. of 72)

Number	Silicone Thickness (MILS)	Teflon® Thickness (MILS)	Fits These Containers
12708	75	10	100 µl Reacti-Vial™ Small Reaction Vials 1.5 ml Screw Cap Septum Vials
12712	90	10	0.3 and 1.0 ml Reacti-Vial™ Small Reaction Vials 2.0 and 3.5 ml Screw Cap Septum Vials
12713	90	10	7 ml Screw Cap Septum Vials
12716	90	10	14 ml Screw Cap Septum Vials 15 ml (1/2 oz.) Screw Cap Bottles
12718	90	10	3 and 5 ml Reacti-Vial™ Small Reaction Vials 10 and 25 ml Reacti-Flask™ Small Reaction Flasks 60 ml (2 oz.) Screw Cap Bottles
12720	125	10	6-125 ml Hypo-Vial™ Sample Storage Vials
12820	90	10	120 ml (4 oz.) Screw Cap Bottles
12722	90	10	25 and 40 ml Screw Cap Septum Vials 240 ml (8 oz.) Screw Cap Bottles 10 ml Reacti-Vial™ Small Reaction Vials

# Teflon®/Rubber Laminated Discs

**For a highly inert and nonreactive seal.**

Teflon®/Rubber Laminated Discs are constructed of white pharmaceutical rubber with 5 mils of Teflon® Coating bonded to one side. Total thickness of the disc is approximately 60 mils. The discs are excellent for use as cap liners when a highly inert nonreactive seal is desired.

Rigid Teflon®/Rubber Laminated Discs are more difficult to puncture than Tuf-Bond™ Discs. Consequently, care must be taken when puncturing these discs to avoid bending the needle.

Teflon®/Rubber Laminated Discs are autoclavable and demonstrate no loss of integrity after heating above 100°C for 5 hours.

## Teflon®/Rubber Laminated Discs (pkg. of 72)

Number	Size	Fits These Containers
12412	12 mm	0.3 and 1.0 ml Reacti-Vial™ Small Reaction Vials, 2.0 and 3.5 ml Screw Cap Septum Vials
12413	13 mm	7 ml Screw Cap Septum Vials, 1 ml Hypo-Vial™ Sample Storage Vials
12418	18 mm	3 and 5 ml Reacti-Vial™ Small Reaction Vials, 10 and 25 ml Reacti-Flask™ Small Reaction Flasks
12422	22 mm	25 and 40 ml Screw Cap Septum Vials, many 8 oz. (1/2 pt.) Bottles

# Mininert® Valves

**Ideal for chemicals that deteriorate or evaporate in conventionally sealed containers.**



They're easy to use. Push the green button to open, insert syringe needle and take sample, withdraw needle, then push red button to close. To change needle-seal septa, simply push the old septum out with a 1/8" diameter rod and

push a new cylinder septum in. This is done with the valve closed to prevent exposure of contents.

Mininert® Push-button Valves are highly dependable leak-tight closures for Screw Cap Septum Vials and other laboratory containers. Constructed of chemical-resistant Teflon® Coating, the valves provide an inert, high-pressure seal.

Mininert® Valves are a superior replacement for rubber septum stoppers and ordinary screw caps. You can easily access the contents by inserting a syringe needle. A rubber gasket above the Teflon® Coating Valve stem provides a seal for the needle when the valve is open. The seal prevents leakage and exposure of the contents during sampling.

**Mininert® Valves are unique and practical seals for these containers:**

- Screw Cap Septum Vials
- Hypo-Vial™ Sample Storage Vials
- Reacti-Vial™ Small Reaction Vials

## Ordering Information

Number	Description	Fits these Containers	Size	Pkg. Size
10135	Mininert® Valves	3 and 5 ml Reacti-Vial™ Small Reaction Vials 10 and 25 ml Reacti-Flask™ Small Reaction Flask	12 mm	12/pkg
10130	Mininert® Valves	25 and 40 ml Screw Cap Septum Vials 5 and 7 ml Tuf-Tainer™ Vials	13 mm	12/pkg

## Septa Compatibility Guide

Closure Type	Resealability	Recommended For Use With	Not Recommended For Use With
Tuf-Bond™ Discs	Excellent	DMF, DMSO, organic solvents, pyridine, THF and silylation reactions	Strong corrosives, such as chlorosilanes
Teflon®/Rubber Laminated Discs	Poor	Corrosives such as chlorosilanes, DMF, DMSO, organic solvents, pyridine and THF	Tritluoroacetic anhydride
Butyl Rubber Septa	Good	Acetic acid, acetic anhydride <50%, acetone, acetonitrile, alcohols, amines, carbon dioxide, diethylamine, DMF, DMSO, ethanolamine ethylacetate (for short-term use), phenol & water	TEA triethylamine, alkanes, benzene, carbon disulfide chlorinated solvents, cyclohexane, ethylacetate for long-term use, fuels, heptane, and hexane
Hycar Septa	Good	Chlorinated organic compounds, hydrocarbons such as heptane and hexane	Acetonitrile, benzene, chloroform, DMF, DMSO, pyridine, THF and toluene
Silicone Septa	Excellent	Acetone, alcohols, DMF, DMSO, ether, some other ketones and water	Acetonitrile, benzene, chloroform, heptane, hexane, pyridine, THF and toluene

# PCC-54® Detergent Concentrate

**Offers safe and effective cleaning in a convenient pump-handle dispenser!**



## PCC-54® Concentrate offers these unique features:

- Rinses clean, providing film-free surfaces
- Safe to handle
- Economical
- Trouble-free disposal

PCC-54® Concentrate is a special detergent formulation designed for efficient cleaning of glass, plastic, porcelain and ferrous metal surfaces. PCC-54® Concentrate can be used in all applications in which ultimate cleanliness of apparatus and equipment is essential. It removes stubborn contaminants and rinses completely, leaving your surface free of any annoying residues or films.

PCC-54® Concentrate is an effective replacement for chromic acid or other harsh cleaning solutions – eliminating harm to you, your clothing or your apparatus (see comparison chart below).

Only Pierce offers PCC-54® Concentrate in an exclusive three-liter pump dispenser bottle. Sparkling clean labware is just a pump away.

## Here's how easy sparkling clean labware can be (instructions for normal use):

1. Pump 20 cc of PCC-54® Concentrate and add to 1 liter of water at 50°C.
2. Immerse object completely into solution.
3. Incubate at least 30 minutes.
4. Immediately after cleaning, rinse with tap water, then with distilled water.

## Comparison of Pierce Cleaning Agents to Chromic Acid for Effectiveness and Safety

PCC-54® & RBS CLEANING AGENTS	CHROMIC ACID
Moderately alkaline	Strongly acidic
Harmless to skin and clothing	Extremely corrosive to skin and clothing
No disposal problem; Causticity is the same as .001 N sodium hydroxide solution	Disposal difficulties due to corrosive nature
No etching of glassware	Frequently etches glassware
Rinses free at all residue	Occlusion of chromic salts
Works rapidly when boiled; If using cold solution, action can be accelerated by increasing concentration	Slow acting
Acts by solubilizing and emulsifying contaminants with no destructive chemical action likely to yield gaseous toxic radioactive compounds	Cleans by oxidation. Compounds containing radioactive Cl, F, S, N or C atoms may yield radioactive gases, producing respiratory hazards
Easily removes difficult substances such as tars, distillation residues, silicone, oils, etc.	Has only a slight effect on these materials

## Ordering Information

Number	Description	Quantity	Volume 12% Solution
72288	PCC-54® Detergent Concentrate	3 liters	150 liters
72289	PCC-54® Detergent Concentrate	4 x 3 liters	600 liters
72290	PCC-Free™ Phosphate Free Detergent Concentrate	3 liters	150 liters

# RBS® Cleaning Agents

**This versatile, safe alternative to chromic acid even inactivates the HIV virus!**

## Useful for:

- General laboratory work
- Optical applications – slides, lenses, mirrors, reflectors, etc.
- Radiochemical decontamination
- Food and beverage equipment sanitation
- Electronics – ultimate cleanliness without residue



RBS® Cleaning Agents are mildly basic surfactant concentrates that will not harm you or your clothing. They are a mixture of anionic and nonionic detergents capable of removing both organic and inorganic materials. Glass, plastics, quartz, porcelain and ferrous metal apparatus can be cleaned safely and effectively in a 2% working solution of the concentrate or in a 0.2% solution of the solid. These easy-to-use cleaning agents work by penetrating contaminated substances and the surface to be cleaned.

Because RBS® Cleaning Agents are nonflammable and noncorrosive, they are easy to handle and can be disposed of safely. The table on page 75 compares Pierce cleaning agents with the

effective, but hazardous, chromic acid cleaning solution. Pierce cleaning agents are superior to chromic acid in every category.

RBS® Cleaning Agents prepared with ethanol or methanol are safer for both the labware and the user than the caustic alcohol solutions commonly used to remove tars, distillation heels and silicone greases. The alcohol/RBS® Agent combination removes tenacious substances from glassware without worry about caustic burns or etching of the glass surfaces.

The Pasteur Institute of Paris, France demonstrated that RBS®-35 Detergent even inactivates the HIV virus. The virus was inactivated after 5 minutes in contact with a 4% solution of the RBS®-35 Detergent.

RBS® Cleaning Agents are available in a variety of forms to meet your cleaning needs.

**RBS®-35 Detergent Concentrate** is a biodegradable, general purpose lab cleaner that allows for long soaking without leaving deposits. It decontaminates radioactivity and removes protein, lipids, greases, oils and still residues.

In the past, pipettes and other laboratory equipment with hard-to-clean crevices have been soaked in hazardous chemicals such as chromic acid. RBS®-35 Detergent Concentrate offers a safe, effective alternative to chromic acid for these applications (see table on page 75).

**RBS® Detergent Solid** is a low-foaming, surface-active powder that is ideal for use in washing machines equipped with a powder dispenser. It is easily eliminated by rinsing, and it leaves no traces. Recommended concentration is 0.1-0.3%.

**RBS®-pF Detergent Concentrate** was developed as a substitute for surfactants that contain phosphates. It is biodegradable and contains no phosphates, which can harm the environment when released into sewage systems.

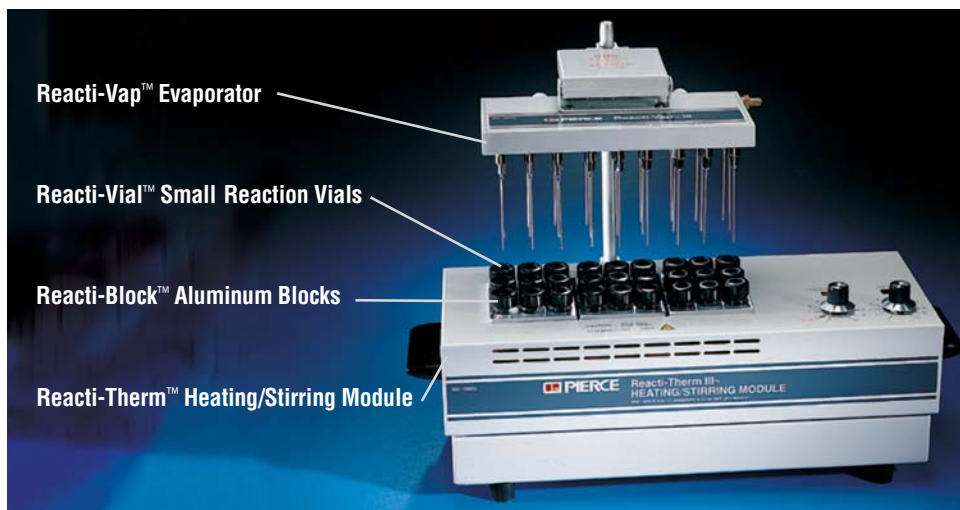
## Ordering Information

Product #	Description	Quantity	Volume (2% Solution)
27950	RBS®-35 Concentrate	1 kg	50 qt.
27952	RBS®-35 Concentrate	5 kg	200 qt.
27853	RBS®-35 Concentrate	30 kg	1,200 qt.
27968	RBS® Solid	5 kg	
27959	RBS®-pF* Concentrate	1 kg	50 qt.
27960	RBS®-pF* Concentrate	5 kg	200 qt.

\* pF = phosphate free

# Reacti-Therm™ Dry Block Sample Incubation System

Featuring our exclusive Reacti-Therm™ Modules for dry block heating - and no sample contamination!



The Reacti-Therm™ System delivers uniform dry heat with unmatched convenience and versatility. The dry heat prevents many of the problems associated with water baths, including sample contamination.

**Reacti-Therm™ Modules** are easy-to-use, constant-temperature heaters that are ideal for your routine incubations. They also provide constant temperature control for samples held in our Reacti-Vial™ Small Reaction Vials, as well as for samples in test tubes, microcentrifuge tubes and other small containers. Most applications that require heating, stirring or evaporation of small samples would benefit from the convenience and efficiency of Reacti-Therm™ Modules. These applications include:

- Sample incubation
- Sample evaporation
- Protein hydrolysis
- Small-scale reactions
- Vacuum hydrolysis for amino acid analysis
- Derivatization reactions for HPLC and GC

Reacti-Therm™ Modules transfer heat through an aluminum alloy block. They hold a wide variety of interchangeable Reacti-Block™ Aluminum Blocks (page 79). Choose from four module designs to meet your exact incubation needs.

**Single-Block Reacti-Therm™ Heating Modules and Reacti-Therm™ Heating/Stirring Modules – now quicker than ever!**

Single-block Reacti-Therm™ Heating Modules feature a solid state electronic control. This highly efficient control system allows faster and easier temperature settings.

PIERCE

Sample Handling

## Single-Block Reacti-Therm™ Features:

- Holds one Reacti-Block™ Aluminum Block to efficiently meet small capacity requirements
- Temperature range ambient -10°C to 150°C
- Temperature controlled  $\pm 0.5^\circ\text{C}$
- Variable-speed stirring with Reacti-Therm™ Heating/Stirring Module, Product #s 19870 (110 v) and 18971 (220 v). Features a built-in stirrer that is ideal for promoting smooth, uniform reactions and dissolving sticky residues
- Easy-to-use units include complete instructions

## Reacti-Therm™ III Module – triple the sample capacity!

- Holds three Reacti-Block™ Aluminum Blocks for larger capacity incubation requirements
- Temperature range ambient -10°C to 200°C – ideal for high temperature reactions
- Temperature controlled  $\pm 0.5^\circ\text{C}$  at 37°C for incubation
- Variable-speed stirring with Reacti-Therm™ III Heating/Stirring Module, Product #s 18935 (110 v) and 18940 (220 v)
- Complete easy-to-follow instructions are included with every unit

## 110 Volt Modules

Number	Description	Pkg. Size
18870	Reacti-Therm™ Heating Module (Single Block)	110 v
18970	Reacti-Therm™ Heating/Stirring Module (Single Block)	110 v
18835	Reacti-Therm™ III Heating Module (Triple Block)	110 v
18935	Reacti-Therm™ III Heating/Stirring Module (Triple Block)	110 v

## 220 Volt Modules

Number	Description	Pkg. Size
18970	Reacti-Therm™ Heating Module (Single Block)	220 v
18971	Reacti-Therm™ Heating/Stirring Module (Single Block)	220 v
18840	Reacti-Therm™ III Heating Module (Triple Block)	220 v
18940	Reacti-Therm™ III Heating/Stirring Module (Triple Block)	220 v

*Underwriters Laboratories, Inc. Listed*

*Note: Our 220-volt modules bear a CE marking for meeting the requirements of the European Union's Low-Voltage and EMC Directives.*

# Reacti-Block™ Aluminum Blocks

**There is one that is right for your sample needs!**













Reacti-Block™ Aluminum Blocks are available with many hole configurations, machine-drilled to accommodate almost any size Reacti-Vial™ Small Reaction Vial (page 68), test tube or microcentrifuge tube. These highly efficient

units are constructed of an aluminum alloy for optimal thermal conductivity. To ensure proper heat transference, be sure to have a close block-to-sample container fit.

Each Reacti-Block™ Aluminum Block contains a thermometer well 7.1 mm dia. x 36.5 mm deep (excluding blank block J and K). Block dimensions are 9.4 cm W x 7.5 cm D x 5.1 cm H for all blocks except F, G and J. Dimensions for these are 9.4 cm W x 7.5 cm D x 7.6 cm H.

The following Reacti-Block™ Aluminum Blocks can be used with all Reacti-Therm™ Modules including those equipped with Reacti-Vap™ Evaporators. Blocks B-1 and T-1 are specifically designed for use with Reacti-Vap™ Units.






## Ordering Information

Number	Description
18801	 <b>Reacti-Block™ A-1</b> Holds 13 x 0.3 ml or 1 ml Reacti-Vials™; 13 holes/14 mm dia. x 23 mm deep
18802	 <b>Reacti-Block™ B-1</b> Holds 9 x 3 ml or 5 ml Reacti-Vials™; 9 holes/21 mm dia. x 32 mm deep
18803	 <b>Reacti-Block™ C-1</b> Holds 13 x 3.5 ml Screw Cap Septum Vials; 13 holes/15 mm dia. x 34 mm deep
18804	 <b>Reacti-Block™ D-1</b> Holds 13 x 1 ml Hypo-Vials™; 13 holes/15 mm dia. x 21 mm deep
18811	 <b>Reacti-Block™ M-1</b> Holds 6 x 27.5 ml Reacti-Vials™; 6 holes/28.5 mm dia. x 70 mm deep
18814	 <b>Reacti-Block™ Q-1</b> Holds 8 x 10 ml Reacti-Vials™; 8 holes/26 mm dia. x 46 mm deep
18816	 <b>Reacti-Block™ S-1</b> Holds 13 x 13 mm dia. Test Tubes; 13 holes/14 mm dia. x 45 mm deep
18817	 <b>Reacti-Block™ T-1</b> Holds 9 x 16 mm dia. Test Tubes; 9 holes/17 mm dia. x 45 mm deep
18818	 <b>Reacti-Block™ U-1</b> Holds 8 x 20 mm dia. Test Tubes; 8 holes/21 mm dia. x 45 mm deep
18819	 <b>Reacti-Block™ V-1</b> Holds 17 Microcentrifuge Test Tubes; 17 holes/11 mm dia. x 45 mm deep

## Reacti-Block™ Aluminum Blocks continued

The Reacti-Block™ Aluminum Blocks featured below are designed to be used exclusively with the Reacti-Therm™ Modules. They do not fit Reacti-Vap™ Evaporators.

### Ordering Information

Number		Description
18806		<b>Reacti-Block™ F</b> Holds 8 x 5 ml or 8 ml Vacuum Reaction Tubes; 8 holes/10 mm dia. x 64 mm deep
18807		<b>Reacti-Block™ G</b> Holds 4 x 20 ml Vacuum Reaction Tubes; 4 holes/19 mm dia. x 64 mm deep
18809		<b>Reacti-Block™ J</b> Blank/no holes (for custom drilling) 3" deep
18810		<b>Reacti-Block™ K</b> Blank/no holes (for custom drilling) 2" deep
18812		<b>Reacti-Block™ L</b> Holds 16 x 0.1 ml Reacti-Vial™ Vials; 16 holes/12 mm dia. x 21 mm deep

## Reacti-Therm™ Thermometers

**Teflon®-coated, designed specifically for dry incubations.**

### Ordering Information

Number	Description
18914	<b>Reacti-Therm™ Thermometer, Mercury-free (0-100°C)</b>
18915	<b>Reacti-Therm™ Thermometer, Mercury-free (0-200°C)</b>

**To complete your Reacti-Therm™ System Order:**

1. Reacti-Therm™ Module
2. Reacti-Block™ Aluminum Block(s)
3. Reacti-Therm™ Thermometer
4. Reacti-Tap™ Evaporator

# Reacti-Vap™ Evaporators

**Sample evaporation made easy!**



Reacti-Vap™ Evaporator (9-port) and Reacti-Vap™ III Evaporator (27-port).

Reacti-Vap™ Evaporators are precision-machined gassing manifolds. They provide simple, efficient evaporation by allowing the simultaneous or separate delivery of nonreactive pressurized gas to samples.

The Reacti-Vap™ III Evaporator triples the number of samples you can evaporate. Nine needles attach to each of the three individually regulated chambers. The evaporating head tilts back for easy needle attachment and removal.

The standard Reacti-Vap™ Evaporator attaches easily to single-block Reacti-Therm™ Modules. The Reacti-Vap™ III unit attaches easily to Reacti-Therm™ III Modules.

## Ordering Information

Number	Description
18780	<b>Reacti-Vap™ Evaporator (9-port)</b> For use with Reacti-Therm™ Single Block Modules; 18870, 18790, 18970 and 18971 Includes 9 needles and plugs
18785	<b>Reacti-Vap™ III Evaporator (27-port)</b> For use with Reacti-Therm™ III Modules; 18835, 18840, 18935 and 18940 Includes 27 needles and plugs

## Reacti-Vap™ Standard and Teflon® Coated Needles

**Reduce cross-contamination and corrosion.**



Reacti-Vap™ Teflon® Coated Needles are made exclusively for use in Reacti-Vap™ Evaporators. They are blunt-ended, 19-gauge, stainless steel needles that reduce cross-contamination and corrosion when evaporating solvents that contain strong acids.

Each Reacti-Vap™ Needle has a Luer-Lok® hub for leak-proof attachment to Reacti-Vap™ Evaporators. Needles are available in 4- and 6-inch lengths.

## Ordering Information

Product #	Description	Pkg. Size
18784	<b>Reacti-Vap™ Teflon® Coated Needles</b> 4-inch (102 mm) x 19 gauge	Pkg. of 9
18786	<b>Reacti-Vap™ Teflon® Coated Needles</b> 6-inch (152 mm) x 19 gauge	Pkg. of 9
18782	<b>Reacti-Vap™ Replacement Tube Kit</b> 2.5 inch (64 mm)	Pkg. of 9 needles and plugs

# Reacti-Vial™ Magnetic Stirrers

**Offer faster reaction times with smooth mixing of small samples.**



Mounted on a triangular matrix, these small Teflon®-coated stirring bars fit the cone portion of 0.3, 1.0, 3.0, 5.0 and 10.0 ml Reacti-Vial™ Small Reaction Vials. For more information on Reacti-Vial™ Small Reaction Vials, see page 68.

**When used with Reacti-Therm™ Heating/Stirring Modules, these efficient stirrers provide**

- Faster reaction times with smooth, efficient mixing of small reaction samples
- Solubilization of sticky concentrated residues such as those found on evaporation of sugar solutions
- Increased speed-of-surface reactions by keeping insoluble reactants in suspension

## Ordering Information

Product #	Description	Pkg. Size
16000	<b>Reacti-Vial™ Magnetic Stirrers</b> For use with 3.0, 5.0 and 10 ml Reacti-Vial™ Small Reaction Vials	Pkg. of 6
16010	<b>Reacti-Vial™ Magnetic Stirrers</b> For use with 0.3 and 1.0 ml Reacti-Vial™ Small Reaction Vials	Pkg. of 6

# Vacuum Hydrolysis Tubes

**Completely reusable and compatible with Reacti-Therm™ Modules.**



**Applications:**

- Hydrolysis
- Sample concentration
- Lyophilization
- Hydrazinolysis





## Ordering Information

Product #	Description	Pkg. Size
29550	<b>Vacuum Hydrolysis Tube</b> , 8 mm x 60 mm	Each
29560	<b>Vacuum Hydrolysis Tube</b> , 10 mm x 100 mm	Each
29564	<b>Vacuum Hydrolysis Tube</b> , 19 mm x 100 mm	Each

# Reacti-Block™ Aluminum Blocks For Vacuum Reaction Tubes

For use with Reacti-Therm™ Heating/Stirring Modules.

## Ordering Information

Number		Description
18806		<b>Reacti-Block™ F</b> Holds 8 x 5 ml or 8 ml Vacuum Reaction Tubes; 8 holes/10 mm dia. x 64 mm deep
18807		<b>Reacti-Block™ G</b> Holds 4 x 20 ml Vacuum Reaction Tubes; 4 holes/19 mm dia. x 64 mm deep
18809		<b>Reacti-Block™ J</b> Blank/no holes (for custom drilling) 3" deep
18810		<b>Reacti-Block™ K</b> Blank/no holes (for custom drilling) 2" deep

## Hydrochloric Acid (6 N)

**Ready-to-use reagents in convenient packaging.**

Pierce Hydrochloric Acid is purified and packaged to ensure a ninhydrin negative blank on hydrolysis. Convenient, pre-scored ampule packaging of the ready-to-use HCl maintains reagent integrity. This virtually eliminates exposure to laboratory atmospheres, fingerprints and other contaminants resulting from pipetting from bulk bottles.

An excellent description of the total protein hydrolysis technique using constant boiling hydrochloric

acid is detailed by Eveleigh and Winter.<sup>1</sup> With constant boiling hydrochloric acid, tryptophan losses are expected. Standard protein hydrolysis conditions are 105-110°C for 16-24 hours. At 150°C, this reagent can be used for the rapid (six-hour) hydrolysis of peptides.

### Reference

1 Eveleigh, J.W. and Winter, G.D. (1970). *Protein Sequences* Ed. Needleman, S.B., Springer-Verlag, pp. 92-95.

## Ordering Information

Product #	Description	Pkg. Size
✘ 24308	<b>Hydrochloric Acid</b> Constant Boiling, (6 N), Sequanal Grade	10 x 1 ml ampules

✘ Additional hazardous handling charge.

# Alphabetical Index

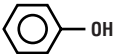
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# Derivatization Reagents for Specific Functional Groups

Functional Group	Procedure	Reagent	Derivative	Notes	
<b>Amides</b> $\begin{array}{c} \text{O} \\    \\ \text{-C-NH}_2 \\ \text{Primary} \end{array}$  $\begin{array}{c} \text{O} \\    \\ \text{-C-NHR} \\ \text{Secondary} \end{array}$	Silylation	BSA	TMS Amides	Difficult to form due to steric hindrance	
		BSTFA	TMS Amides		
		BSTFA+TMCS	TMS Amides		TMCS used as a catalyst
		MSTFA	TMS Amides		Reaction byproducts more volatile
		MSTFA+TMCS	TMS Amides		
	Acylation	Tri-Sil <sup>®</sup> Reagents	TMS Amides		
		MTBSTFA	TBDMCS Amides	Difficult to form; very stable	
		MTBSTFA+TBDMCS	TBDMCS Amides		TBDMCS aids derivatization
		MBTFA	Trifluoroacetamides		
		TFAA	Trifluoroacetamides		
PFAA	Pentafluoropropionamides	Good for ECD detection			
Alkylation	HFBA	Heptafluorobutyamides			
	MethElute <sup>™</sup> Reagent	Methyl Amides	On-column derivatization especially for drugs		
<b>Amines</b> $\begin{array}{c} \text{H} \\   \\ \text{-C-NH}_2 \\   \\ \text{H} \\ \text{Primary} \end{array}$  $\begin{array}{c} \text{H} \\   \\ \text{-C-NHR} \\   \\ \text{H} \\ \text{Secondary} \end{array}$	Silylation	BSA	TMS	TMCS aids derivatization	
		BSTFA	TMS		
		BSTFA+TMCS	TMS		
		MSTFA	TMS		
		MSTFA+TMCS	TMS		
	Acylation	Tri-Sil <sup>®</sup> Reagents	TMS		
		MTBSTFA	TBDMCS	Difficult to form, but more stable	
		MTBSTFA+TBDMCS	TBDMCS		TBDMCS aids derivatization
		MBTFA	Trifluoroacetamides	Good for trace analysis with ECD	
		TFAA	Trifluoroacetamides	Good for trace analysis with ECD	
Alkylation	TFAI	Trifluoroacetamides	Good for trace analysis with ECD		
	PFAA	Pentafluoropropionamides			
	PFPI	Pentafluoropropionamides			
	HFAA	Heptafluorobutyamides			
	HFBI	Heptafluorobutyamides			
<b>Carbohydrates</b> $(\text{CH}_2\text{OH})_n$	Silylation	MethElute <sup>™</sup>	Methyl Amides	On-column derivatization for specific drugs	
		MSTFA	TMS		
		TMSI	TMS	Can be used with some syrups	
	Acylation	Tri-Sil <sup>®</sup> Reagents	TMS		
		MBTFA	Trifluoroacetates	Volatile derivatives of mono-, di- and trisaccharides	
	TFAI	Trifluoroacetates			
	<b>Carboxyl</b> $\begin{array}{c} \text{O} \\    \\ \text{-C-OH} \end{array}$	Silylation	BSA	TMS	Easily formed, generally not stable, analyze quickly
			BSTFA	TMS	
			BSTFA+TMCS	TMS	
			MSTFA	TMS	
TMCS			TMS		
Alkylation		TMSI	TMS	Can be used with some salts	
		Tri-Sil <sup>®</sup> Reagents	TMS		
		MTBSTFA	TBDMCS		More stable than TMS derivatives
		MTBSTFA+TBDMCS	TBDMCS		
		PFBBR	Pentafluorobenzyl Esters		Used in EC detection & UV, MS
BF <sub>3</sub> -Methanol	Methyl Esters	Best for large samples of fatty acids			
<b>Hydroxyl-OH</b> $\text{R-OH}$ Alcohols   Phenols	Silylation	Methyl-8 <sup>®</sup> Reagent	Methyl Esters	Fatty acids and amino acids	
		MethElute <sup>™</sup> Reagent	Methyl Esters	On-column derivatization	
		PFAA+Pentafluoropropanol	Pentafluoropropyl Ester	Drug analysis	
		BSA	TMS	Most often used derivatives	
		BSTFA	TMS		
	BSTFA+TMCS	TMS	Good thermal stability		
	HMDS	TMS	Poor hydrolytic stability		
	MSTFA	TMS	Weak donor usually used with TMCS		
	Alkylation	MSTFA+TMCS	TMS	Weak donor usually used with HMDS; can be used with salts	
		TMCS	TMS		
TMSI		TMS			
Tri-Sil <sup>®</sup> Reagents		TMS			
MTBSTFA		TBDMCS	Can be used with syrups		
Phenols	Silylation	MTBSTFA+TBDMCS	TBDMCS	More stable than TMS, good MS fragmentation patterns	
		MBTFA	TBDMCS		TBDMCS aids derivatization
	Acylation	Trifluoroacetates	Good for trace analysis with EDC		
		TFAA	Trifluoroacetates	Good for trace analysis with EDC	
		TFAI	Trifluoroacetates	Good for trace analysis with EDC	
		PFPI	Pentafluoropropionates	Good for trace analysis with EDC	
		PFAA	Pentafluoropropionates	Good for trace analysis with EDC	
		HFBI	Heptafluorobutrates	Good for trace analysis with EDC	
		HFAA	Heptafluorobutrates	Good for trace analysis with EDC	
		PFBBR	Pentafluorobenzyl Ethers	With alkoxides only	

# PIERCE

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