

Total Protein Assays — Overcoming Interfering Substances

Compat-Able™ Protein Assays

Do you worry about sample components interfering with your protein analysis results?

Pierce BCA™ and Coomassie Plus – The Better Bradford™ Assays, recognized around the world as the best detergent- and reducing agent-compatible assays (respectively) for total protein analysis, are paired with a great sample preparation reagent. These unique reagents dispose of any interfering substances in your sample by selectively precipitating the protein, allowing the nonprotein components to be removed easily. Precipitated protein is recovered in water and assayed with the BCA™ Protein Assay or Coomassie Plus – The Better Bradford™ Assay.



Highlights:

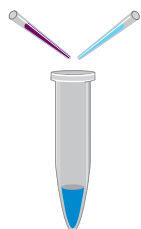
- Ready-to-use sample preparation reagents save time and effort
- Four-step protocol takes less than 10 minutes to complete
- Room temperature-stable sample preparation reagents can be stored on your bench top so they won't get lost in the cold room or hidden in the lab refrigerator
- Precipitates protein out of solution, leaving potentially interfering substances to be decanted away without dialysis or gel filtration, saving time and avoiding sample loss or dilution
- Easily adaptable to pre-treatment of many samples at one time
- Adaptable to both a test tube and microcentrifuge tube sample preparation protocol, to allow for 50 µl or 100 µl sample volumes
- Sample prep reagents are available with the BCA™ or Coomassie Assays or sold separately

Ordering Information

| Product # | Description | Pkg. Size |
|--------------|---|---|
| 23229 | BCA™ Compat-Able™ Protein Assay Kit Contains one each of the following: Product # 23227, BCA Protein Assay Kit <i>Sufficient reagents to perform 250 standard tube assays or 2,500 microplate assays.</i> BCA™ Reagent A BCA™ Reagent B BSA Standards (2 mg/ml) Product # 23215, Compat-Able™ Protein Assay Preparation Reagent Set (see description below) | Kit 2 x 250 ml 25 ml 10 x 1 ml |
| 23239 | Coomassie Plus Compat-Able™ Protein Assay Reagent Kit Contains one each of the following: Product # 23236, Coomassie Plus Protein Assay Reagent Kit <i>Sufficient materials for 630 standard assays, 950 microassays or 3,160 microplate assays.</i> Coomassie Plus Reagent Formulation BSA Standards (2 mg/ml) Product # 23215, Compat-Able™ Protein Assay Preparation Reagent Set (see description below) | Kit 950 ml 10 x 1 ml |
| 23215 | Compat-Able™ Protein Assay Preparation Reagent Set <i>Two-reagent set with sufficient material to pre-treat up to 500 samples prior to total protein assay.</i> Compat-Able™ Protein Assay Preparation Reagent 1 Compat-Able™ Protein Assay Preparation Reagent 2 | Kit 250 ml 250 ml |

Make Almost any Protein Sample Compatible with the BCA™ or Coomassie Plus – The Better Bradford™ Assays in Four Simple Steps

ADD:
Sample and Reagent 1; mix
Add Reagent 2; mix



Centrifuge tubes:
5 minutes at 10,000 x g



Invert tubes:
decant and blot



ADD:
Ultrapure water; mix



Sample is now ready to submit to a BCA™ or Coomassie Plus – The Better Bradford™ Assay

Total Protein Assays — Amine Detection

o-Phthalaldehyde (OPA) Fluorescent Protein Assay

The Pierce Fluoraldehyde Protein/Peptide Assay is an *o*-phthalaldehyde-based reagent developed to detect minute amounts of protein and peptides. Fluoraldehyde reactions are complete in less than one minute with sensitivity down to 50 ng/ml. While some solutions interfere with protein/peptide measurement at 280 nm, the Pierce Fluoraldehyde Assay is compatible with many substances that interfere with other protein assays, such as detergents and reducing agents. Amine-containing buffers must be avoided, however, when performing assays using this chemistry.

In the standard assay mode, the fluoraldehyde ready-to-use formulation can measure protein concentration in the range of 10 to 500 µg/ml, while the micro-assay working range is 50 ng/ml to 25 µg/ml.

The Pierce Fluoraldehyde Protein/Peptide Assay Reagent requires only 200 µl of sample for use in a microplate assay, saving valuable sample and time. Fluoraldehyde assays require an excitation wavelength of 360 nm and emission wavelength of 455 nm.

OPA will react only with primary amines. When reacted with primary amines in the presence of mercaptoethanol, OPA yields an intense blue colored fluorescent product that has a maximum wavelength of excitation of 340 nm and emission at 455 nm.^{1,2} Wavelengths from 330-375 nm have been used for excitation and 436-490 nm for measuring emission. Protein concentrations as low as 50 ng/ml can be measured with an OPA assay. The inherent sensitivity and speed of OPA, along with its broad linear range, makes it a useful protein and peptide assay reagent.

OPA is ideal for assaying peptides that do not contain tyrosine residues, or for other applications in which absorbance at 280 nm cannot be used. Proteins and peptides tested yield linear results over a wide range of concentrations using both standard and microassay protocols.

There is considerable protein:protein and peptide:peptide variation with the OPA assay; therefore, it is best to use a purified sample of the particular protein or peptide as the standard. When this is not possible, the next best option is to use a protein or peptide that gives a response similar to the sample. Alternatively, a commonly accepted standard protein such as bovine serum albumin can be used.

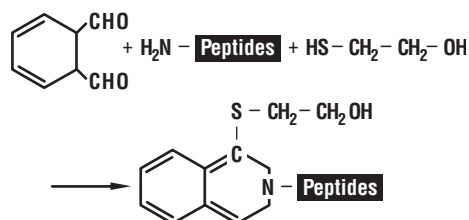


Figure 11. The reaction of *o*-Phthalaldehyde with a primary amine on a peptide in the presence of 2-Mercaptoethanol to form a fluorescently labeled peptide.

Reducing agents and metal chelators do not interfere with an OPA-based assay, provided they are included in the blanks and standards. In addition, most detergents do not interfere. Any common sample buffers and constituents are also compatible, but primary amines such as Tris or glycine buffers will interfere with OPA and must be avoided. Acetylated and other primary amine-blocked peptides will not give a response with OPA.

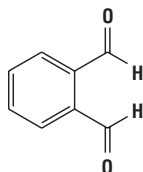
References

1. Ogden, G. and Foldi, P. (1987). *LC•GC* **5** (1), 28-38.
2. Roth, M. (1971). *Anal. Chem.* **43**, 880-882.

Total Protein Assays — Amine Detection [continued]

Fluoraldehyde™ *o*-Phthalaldehyde Crystals

An easy, economical way to detect amino acids in pre- and post-column chromatographic effluents.



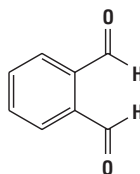
**Fluoraldehyde™
o-Phthalaldehyde**
M.W. 134.13

Highlights:

- Stable in aqueous solution
- Highly sensitive, low background
- Rapid analysis, no heating required

Fluoraldehyde™ *o*-Phthalaldehyde Reagent Solution

Excellent sensitivity — an ideal choice when working with limited amounts of purified protein or peptides.



Fluoraldehyde™ *o*-Phthalaldehyde Reagent Solution
M.W. 134.13
 $\lambda_{ex} = 340 \text{ nm}$
 $\lambda_{em} = 455 \text{ nm}$

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 12) and offers outstanding shelf life (Figure 13). In addition, when compared to other *o*-phthalaldehyde detection reagents, our solution exhibits decreased background over time and a high signal:noise ratio.

Highlights:

- A ready-to-use, highly sensitive fluorescent pre- or postcolumn reagent for amino acid detection and quantitation
- Provides an accurate measure of both composition and absolute protein/peptide content
- Ready-to-use with no processing needed
- Reacts with all primary amine-containing analytes
- High sensitivity; low background

Application Note:

For even greater sensitivity, use a combination of OPA with Fmoc-Chloride with automated pre-column derivatization, detecting both primary and secondary amines. With this application, primary amino acids are first derivatized with OPA, while non-reacted secondary amino acids are then reacted with Fmoc-Chloride, resulting in extraordinary amino acid detection sensitivity and accuracy.^{1,2}

Ordering Information

| Product # | Description | Pkg. Size |
|-----------|--|-----------|
| 26015 | Fluoraldehyde™ <i>o</i> -Phthalaldehyde Crystals | 5 g |

References

- Lindroth, P. and Mopper, K. (1979). *Anal. Chem.* **51**, 1667-1674.
- Lee, K.S. and Drescher, D.G. (1979). *J. Biol. Chem.* **254**, 6248-6251.
- van Eijk, H.M., et al. (1988). *Clin. Chem.* **34**, 2510-2513.
- Graser, T.A., et al. (1985). *Anal. Biochem.* **151**, 142-152.
- Cooper, J.D., et al. (1984). *Anal. Biochem.* **142**, 98-102.
- Krishnamurti, C.R., et al. (1984). *J. Chromatogr.* **315**, 321-331.
- Jones, B.N., et al. (1983). *J. Chromatogr.* **266**, 471-482.
- Lee, H., et al. (1979). *Anal. Biochem.* **96**, 298-307.
- Chen, R.F., et al. (1979). *Biochem. Biophys. Acta* **576**, 440-455.
- Jones, B.N., et al. (1981). *J. Liq. Chrom.* **4**, 565-586.

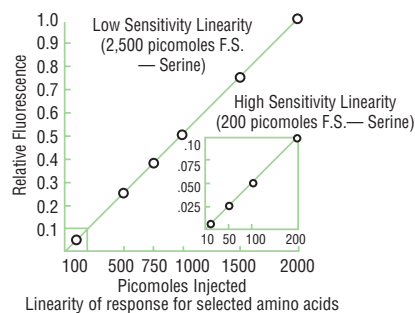


Figure 12. Excellent linear response. Fluoraldehyde™ Reagent Solution shows excellent linear response, whether in the 2,500 or 200 picomole range.

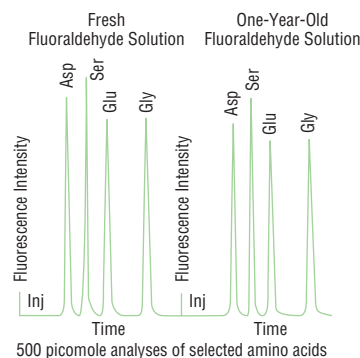


Figure 13. Outstanding shelf life. Comparison of fluorescence response of selected amino acids after reaction with recently prepared and one-year-old Fluoraldehyde™ Reagent Solutions.

Ordering Information

| Product # | Description | Pkg. Size |
|-----------|--|-----------|
| 26025 | Fluoraldehyde™ <i>o</i> -Phthalaldehyde Reagent Solution | 945 ml |

References

- Godel, H., et al. (1992). *LC-GC International* **5**, 44-49.
- Schuster, R. (1988). *J. Chromatogr.* **431**, 271-284.
- Jones, B.N. and Gilligan, J.P. (1983). *American Biotechnology Laboratory*, Dec. Issue, 46-51.
- Benson, J.R. and Woo, D.J. (1984). *J. Chromatogr. Sci.* **22**, 386-399.

Specific Protein Assays — Histidine-tagged Proteins

Histidine-Tagged Protein Detection

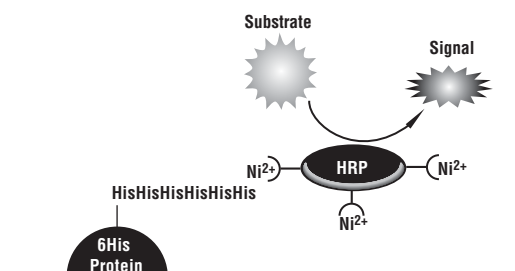
HisProbe™-HRP Western blotting probe takes advantage of the affinity of histidine for the Ni²⁺ cation.

HisProbe™-HRP is a nickel (Ni²⁺)-activated derivative of horseradish peroxidase (HRP). This product has been optimized for direct detection of recombinant histidine-tagged proteins and other histidine-rich proteins. The active ligand is a tridentate chelator that allows Ni²⁺ to be bound in active form for subsequent interaction and detection of target molecules. The active chelator has similar binding capabilities to that reported for iminodiacetic acid, which has long been used for immobilized metal affinity chromatography (IMAC).

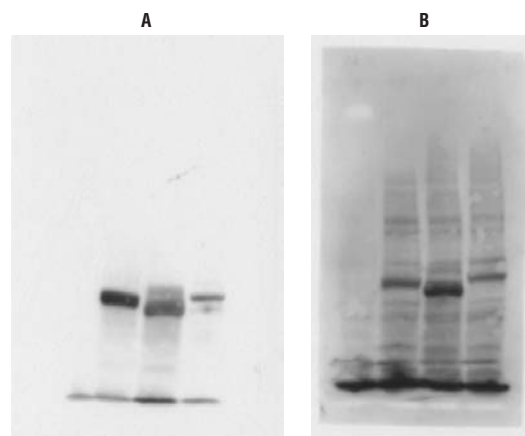
Highlights:

- Yields lower background than anti-histidine antibodies
- Pierce HRP is a high-activity enzyme
- Stripping and reprobing is possible
- HisProbe™-HRP (Ni²⁺) can be used for detection of histidine-tagged proteins

Detection of histidine-tagged fusion proteins with HisProbe™-HRP



Comparison of Polyhistidine-tagged (PHT) Fusion Protein Detection Methods



Panel A using HisProbe™-HRP shows high specific binding and low background. **Panel B** using anti-polyHis failed to recognize two of the three fusion proteins.

Ordering Information

| Product # | Description | Pkg. Size |
|-----------|---|----------------|
| 15165 | HisProbe™-HRP* | 2 mg |
| 15168 | SuperSignal® West Pico HisProbe™ Kit* | Kit |
| | Includes: HisProbe™-HRP* | 2 mg |
| | SuperSignal™ West Pico Chemiluminescent Substrate** | 500 ml |
| | Blocker™ BSA in TBS (10X) | 1 x 125 ml |
| | BupH™ Tris Buffered Saline Packs | 10 x 500 ml |
| | Surfact-Amps® 20 (10%) | 6 x 10 ampules |

References

- Kanaya, E., *et al.* (2001). *J. Biol. Chem.* **276**, 7383-7390.
Steiner, T.S., *et al.* (2000). *J. Clin. Invest.* **105**, 1769-1777.
Guttman, R.P., *et al.* (2001). *J. Neurochem.* **78**, 1083-1093.

Specific Protein Assays — Antibodies

Easy-Titer® IgG and IgM Assay Kits

Simply the fastest, easiest way to quantitate antibodies ... ever!

It is no longer necessary to wait or to rely on inaccurate and insensitive UV or colorimetric IgG determination methods. It is not necessary to struggle with the inadequacies of methods that titrate antibody activity. It is even possible to avoid the tedious, time-consuming ELISA approach to determine antibody titer. Easy-Titer® IgG Assay Kits make it possible to detect IgG in less time and with greater specificity and sensitivity than ever before.

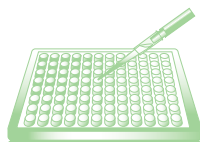
Easy-Titer® Assay Kits do not cross-react with antibodies from other species such as bovine antibodies present in the media used to culture antibody-producing hybridoma cells. This remarkable specificity allows the measurement of human IgG concentrations from a variety of sample types such as culture supernatants, ascites or body fluids without first purifying the antibody from other contaminants.

Highlights:

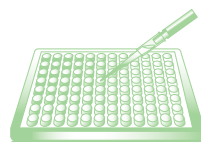
- Easy-to-use particle-based antibody titer determination kit
- Start of assay to recovery of result in less than one hour
- Four times faster than classical ELISA-based protocols
- Convenient design – perform the assay in a 96-well plate and measure the result in a microplate reader
- Measures antibodies from culture supernatants, ascites or body fluids
- Measures humanized antibodies and chimeras with intact Fc regions



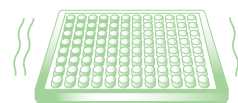
1. Suspend beads



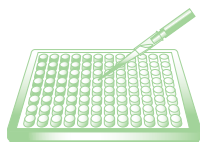
2. Pipette 20 µl beads



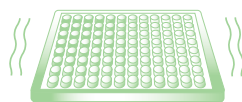
3. Pipette 20 µl sample



4. Incubate on plate mixer;
5 minutes at room temperature



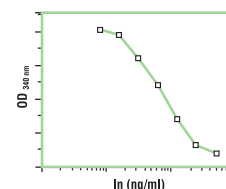
5. Add Blocking Buffer



6. Mix for 5 minutes
on plate mixer



7. Read at 405/340 nm



8. Plot Standard Curve; determine
concentration of Human IgG or
Human IgM

A simple assay makes for an easy-to-perform assay protocol.

Easy-Titer® IgG Assay Kits feature a simple procedure that reduces hands-on time and requires fewer steps that lead to more reproducible results. The entire process can be completed easily in about 30 minutes.

Performance Specifications

Specificity

- Against all IgG subclasses (human, mouse or rabbit)

Sensitivity

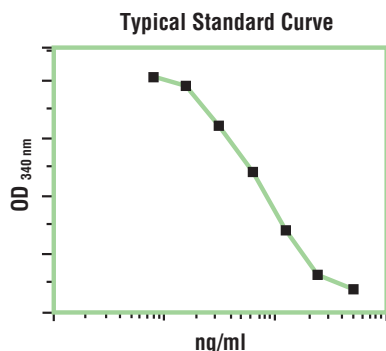
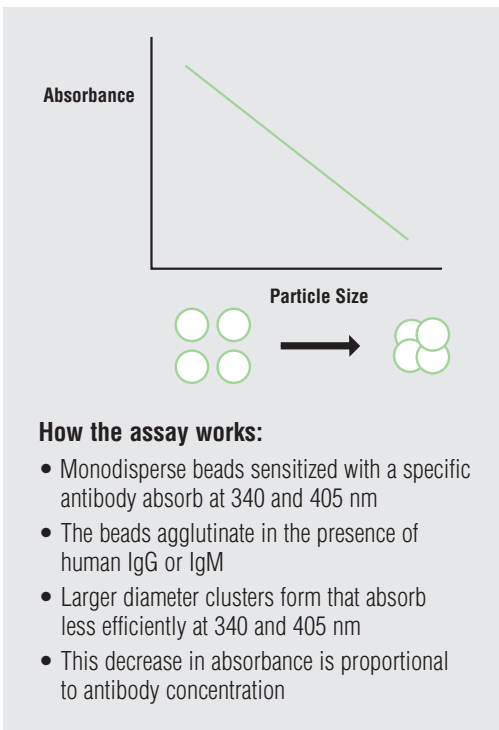
- Detection limit: 15 ng/ml
- Detection range (standard curve): 15 to 300 ng/ml

Coefficient of Variation (intra- and interassay): < 5%

Reaction time: 10 minutes

- Read results at 340 nm or 405 nm

Standard curve calculations are compatible with software supplied for use with microplate readers.



The unknown concentration of IgG is easily determined on a standard curve constructed with serial dilutions of a standard sample

Ordering Information

| Product # | Description | Pkg. Size |
|-----------|--|-----------|
| 23310 | Easy-Titer[®] Human IgG Assay Kit* <i>Sufficient reagents for 96 tests (87 determinations and one standard curve).</i> | Kit |
| | Includes: Goat Anti-Human IgG Sensitized Polystyrene Beads [Monodispersed, polystyrene IgG (Fc) sensitized beads are supplied suspended in a phosphate buffer, pH 7.4 and stabilized with BSA and 0.1% sodium azide] | 2 ml |
| | Easy-Titer [®] Dilution Buffer | 30 ml |
| | Easy-Titer [®] Blocking Buffer | 15 ml |
| 23315 | Easy-Titer[®] Human IgM Assay Kit* | Kit |
| | Includes: Goat Anti-Human IgM Sensitized Beads | 2 ml |
| | Easy-Titer [®] Dilution Buffer | 30 ml |
| | Easy-Titer [®] Blocking Buffer | 15 ml |
| 23300 | Easy-Titer[®] Mouse IgG Assay Kit* | Kit |
| | Includes: Goat Anti-Mouse IgG Sensitized Beads | 2 ml |
| | Easy-Titer [®] Dilution Buffer | 30 ml |
| | Easy-Titer [®] Blocking Buffer | 15 ml |
| 23305 | Easy-Titer[®] Rabbit IgG Assay Kit* | Kit |
| | Includes: Goat Anti-Rabbit IgG Sensitized Beads | 2 ml |
| | Easy-Titer [®] Dilution Buffer | 30 ml |
| | Easy-Titer [®] Blocking Buffer | 15 ml |

***Note: An IgG or IgM Standard is not included in these kits.**

Select the appropriate standard from the Related Pierce Products listed below.

Related Pierce Products:

IgG Standards for Easy-Titer[®] Kits

| Product # | Description | Pkg. Size |
|-----------|-----------------------------------|-----------|
| 31154 | Human IgG, Whole Molecule | 10 mg |
| 31146 | Human IgM, Whole Molecule | 2 mg |
| 31204 | Mouse IgG, Whole Molecule | 10 mg |
| 31235 | Rabbit IgG, Whole Molecule | 10 mg |

Microplate Accessories

| Product # | Description | Pkg. Size |
|-----------|--|------------|
| 15041 | Reacti-Bind[™] 96-Well Plates Corner Notch | 100 plates |
| 15031 | Reacti-Bind[™] 8-Well Strip Plates Corner Notch <i>Includes one strip well ejector per package.</i> | 100 plates |

Reference

Brown, M.A., et al. (2000). *J. Biol. Chem.* **275**, 19795-19802.

Specific Protein Assays — Proteases

QuantiCleave™ Protease Assay Kits – Colorimetric and Fluorometric

Detects protease as low as 2 ng/ml in less than one hour!

QuantiCleave™ Protease Assay Kits are an ideal choice for performing routine assays necessary during the isolation of proteases, or for identifying the presence of contaminating proteases in protein samples. These Protease Assays are also ideal for studying pH or temperature vs. activity profiles of purified proteases.

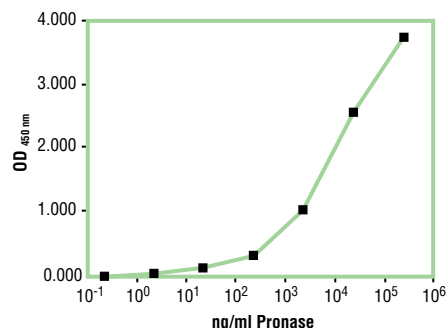
Highlights:

- No corrosive precipitants used
- Entire assay can be run in microplates
- 1,000 times more sensitive, three times faster and uses half the sample of unmodified casein-based protease assays
- Total elapsed time to result – less than one hour
- Measure multiple samples simultaneously in ELISA plate readers
- Time/temperature/pH easily manipulated to optimize sensitivity

The colorimetric QuantiCleave™ Protease Assay Kit uses fully succinylated casein as substrate for this assay. Hydrolysis of this readily soluble casein substrate in the presence of protease results in the release of peptide fragments with free aminoterminal groups. Evidence of protease activity is obtained by reaction of these peptides with trinitrobenzene sulfonic acid (TNBSA), followed by measurement of the absorbance increase that is due to the formation of yellow colored TNB-peptide adducts. A standard protease is provided, allowing you to determine the concentration of protease in samples undergoing analysis.

The QuantiCleave™ Fluorescent Protease Assay Kit is based on a FITC-labeled casein. This sensitive assay can be used in either FRET or FP modes. For more information, refer to the Pierce web site.

Sensitivity of the Colorimetric QuantiCleave™ Protease Assay



Ordering Information

| Product # | Description | Pkg. Size |
|--|---|-----------------------|
| 23263 | QuantiCleave™ Protease Assay Kit | Kit |
| | <i>Sufficient material for 250 assays.</i> | |
| | Includes: Succinylated Casein | 5 x 10 mg |
| | (supplied as a lyophilized salt-free powder) | |
| | 2,4,6-Trinitrobenzene sulfonic acid (TNBSA) | 2 ml |
| TPCK Trypsin standard (40 BAEE units/mg) | 50 mg | |
| BupH™ Borate Buffer Pack (makes 500 ml) | 1 pack | |
| 23266 | QuantiCleave™ Fluorescent Protease Assay Kit | Kit |
| | <i>Sufficient material for at least 1,000 assays in a 96-well format.</i> | |
| | Includes: FITC-Casein, Lyophilized | 2.5 mg |
| | TPCK Trypsin | 50 mg |
| BupH™ Tris Buffered Saline | 1 pack | |
| 23267 | FITC-Casein | 2.5 mg (1,000 assays) |

Reference

Rao, S.K., et al. (1997). *Anal. Biochem.* **250(2)**, 222-227.

Specific Protein Assays — Glycoproteins

Glycoprotein Carbohydrate Estimation Kit

Direct approach to the estimation of carbohydrate content in proteins.



Highlights:

- Enables quick and easy identification of an unknown protein sample as a glycoprotein
- Estimates the percent carbohydrate content of a glycoprotein when run against a set of glycoprotein standards with known carbohydrate content
- Complementary to electrophoresis, Western blotting and ELISA-based procedures often used to detect glycoprotein
- Determines carbohydrate content in three easy steps: (1) oxidize, (2) react and (3) read
- Entire assay performed in less than 75 minutes
- All you need is this kit, a microplate and a plate reader to determine carbohydrate content

Ordering Information

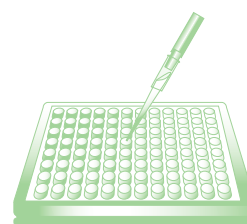
| Product # | Description | Pkg. Size |
|--------------|---|--|
| 23260 | Glycoprotein Carbohydrate Estimation Kit <i>Sufficient reagents for 250 microplate assays or 60 standard test tube assays.</i> Includes: Sodium <i>meta</i> -Periodate Glycoprotein Detection Reagent Glycoprotein Assay Buffer Negative Controls: Lysozyme and BSA Positive Controls: Ovalbumin Apo-Transferrin Fetuin α_1 -Acid Glycoprotein | Kit 500 mg 500 mg 250 ml 2.5 mg each 2.5 mg 2.5 mg 0.25 mg 0.25 mg |
| 23259 | Lyophilized Glycoprotein Standards Set Includes: Negative Controls: Lysozyme and BSA Positive Controls: Ovalbumin Apo-Transferrin Fetuin α_1 -Acid Glycoprotein | Set 2.5 mg each 2.5 mg 2.5 mg 0.25 mg 0.25 mg |
| 23262 | Glycoprotein Detection Agent | 1 g |

Assay Principle

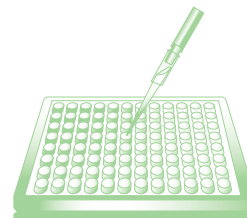
The protein sample under analysis is oxidized and reacted with the exclusive Glycoprotein Detection Reagent. The resulting colored complex is read at 550 nm. From the absorbance of the resulting complex at 550 nm the approximate percentage of carbohydrate in the glycoprotein under analysis can be estimated.

The Phosphoprotein Phosphate Estimation Assay: Microplate Protocol

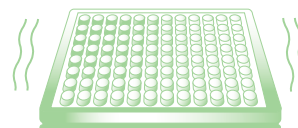
1. Add 50 μ l of protein standard or sample to each well.



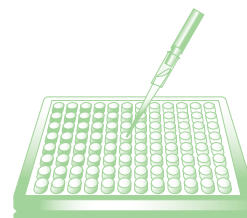
2. Add 25 μ l of 10 mM Sodium *meta*-Periodate in assay buffer.



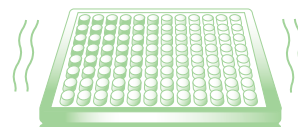
3. Mix and incubate for 10 minutes at room temperature (RT).



4. Add 150 μ l of a 0.5% solution of Pierce Glycoprotein Detection Reagent in 1.0 M NaOH.



5. Mix and incubate at RT for 60 minutes.



6. Read the plate in a microplate reader at 550 nm. Interpolate the results of the unknown with the results of the standard proteins.



Specific Protein Assays — Phosphoproteins

Phosphoprotein Phosphate Estimation Kit

Get some basic questions your target protein answered without having to wait on a Western blot to tell you.



Pierce introduces a novel protein characterization tool that gives today's protein analyst the ability to quickly and reliably determine whether a purified target protein is phosphorylated and, if so, the extent of phosphorylation compared to a phosphoprotein of known phosphorus content. This easy-to-perform assay is specific for estimating phosphoserine or phospho-threonine post-translational modifications and has been adapted to both a tube and convenient microplate format. The Phosphoprotein Phosphate Estimation Assay provides answers that a traditional Western blot simply cannot, and you can get answers about five times faster, too.

Unique advantage of the assay chemistry

The specificity of this assay toward seryl and threonyl phosphate ester modifications can indirectly "detect" a phosphotyrosine modification should the result of the assay be negative. A negative result on a pure protein preparation can suggest that the protein is not phosphorylated or that the protein is, in fact, phosphorylated, but modified by way of the tyrosyl side chains. Further Western blot analysis can verify which conclusion is correct.

In addition, the Phosphoprotein Phosphate Estimation Assay Kit can also be used to determine the amount of a purified known phosphoprotein in a sample. A standard curve can be constructed using a purified preparation of the known protein.

Highlights:

- Easy-to-prepare working reagent
- Colorimetric detection
- Use as qualitative or semi-quantitative assay
- Test tube or microplate assay option
- Estimate extent of phosphoserine/phosphothreonine modification
- Calculate the moles of phosphate (phosphorus) per mole of purified protein
- Use as quantitative assay for known pure phosphoproteins
- Results in about one hour
- Room temperature stability of kit components — saves refrigerator and freezer space

Assay Principle

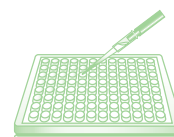
The Phosphoprotein Phosphate Estimation Assay is based on the alkaline hydrolysis of phosphate from seryl and threonyl residues in phosphoprotein and the quantification of the released phosphate by the use of malachite green and ammonium molybdate.

The Phosphoprotein Phosphate Estimation Assay: Microplate Protocol

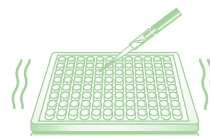
3 parts Malachite Green Solution + 1 part Ammonium Molybdate Solution



1. Mix the Phosphate Reagent Working Solution.



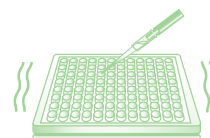
2. Add 50 μ l of protein sample and diluted standards to each well.



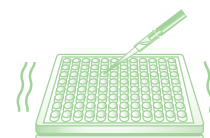
3. Add 50 μ l of 2 N NaOH per well. Mix the plate for 30 seconds.



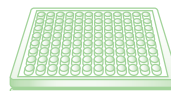
4. Incubate in a 65°C incubator for 30 minutes.



5. Add 50 μ l of 4.7 N HCl per well. Mix for 30 seconds.



6. Add 50 μ l of Phosphate Reagent per well. Mix for 30 seconds.



7. Incubate for 30 minutes at room temperature.



8. Read the plate in a microplate reader at 650 nm. Plot the results of the unknown against the results of the standard protein provided. Calculate the approximate number of phosphorylation sites.

Ordering Information

| Product # | Description | Pkg. Size |
|-----------|--|--|
| 23270 | Phosphoprotein Phosphate Estimation Kit Sufficient reagents for 20 x 96-well microplate assays or 500 test tube assays. Includes: Ammonium Molybdate Solution Malachite Green Solution Phosvitin Positive Control BupH™ Tris Buffered Saline | Kit 25 ml 75 ml 1 mg 1 pkg |

Related Pierce Products:

| Product # | Description | Pkg. Size |
|-----------|---|-----------|
| 24550 | GelCode® Phosphoprotein Staining Kit | Kit |

Phosphoprotein Detection Reagent and Kit

Novel chemistry enables specific detection of phosphorylated protein.

PhosphoProbe™-HRP is an iron (Fe^{3+})-activated derivative of horseradish peroxidase (HRP). PhosphoProbe™-HRP exhibits two distinct binding specificities, one of which is phosphate (R-PO_3)-specific. The other binding specificity is related to a carboxyl-containing binding motif that is common to most proteins and some peptides. This carboxyl motif binding specificity can be used in a total protein detection application. A novel treatment, termed Reactive Chemical Blocking (RCB) using EDC and ethylenediamine, may be used to eliminate this carboxyl-binding motif, thus imparting exclusive specificity toward phosphate groups. PhosphoProbe™-HRP, in conjunction with RCB, is a universal phosphate detection probe. PhosphoProbe™-HRP has been optimized for direct detection of phosphoester molecules such as nucleotides or protein/peptides containing phosphoserine, phosphothreonine and phosphotyrosine.

Ordering Information

| Product # | Description | Pkg. Size |
|-----------|--|--------------------------------------|
| 15166 | PhosphoProbe™-HRP | 2 mg |
| 23031 | Ethylenediamine Dihydrochloride | 10 g |
| 22980 | EDC | 5 g |
| 22981 | EDC | 25 g |
| 15167 | Phosphorylated Protein Detection Kit Includes: PhosphoProbe™-HRP EDC Ethylenediamine Tween®-20 | Kit 2 mg 5 g 10 g 1 vial |

Peroxide Assay

PeroXOquant™ Quantitative Peroxide Assay Kits

Quickly measure peroxide contamination in various biological samples.

Highlights:

- Fast and easy to use
- Peroxidase independent
- No lipid extraction necessary
- Spectrophotometric analysis
- No heating required

PeroXOquant™ Quantitative Peroxide Assays are the simplest assays for detecting the presence of peroxides in both aqueous and lipid-containing laboratory reagents. The basis of these assays is the complexing of ferric ion (Fe^{2+}) by H_2O_2 in the presence of xylenol orange. Peroxides in the sample oxidize Fe^{2+} to Fe^{3+} , and the Fe^{3+} will form a colored complex with xylenol orange that can be read at 560 nm.

The presence of hydrogen peroxide (H_2O_2) can now be detected to monitor any peroxide contamination that may be harmful to biological samples. When performed on a routine basis, the Pierce PeroXOquant™ Quantitative Peroxide Assay can prevent inadvertent introduction of peroxides into your valuable samples. If the effects of peroxide cannot be avoided in a particular system, these assays will help you assess the risk to your sample.

References

- Coutant, F., et al. (2002). *J. Immunol.* **169**, 688-1695.
Goyer, A., et al. (2002). *Eur. J. Biochem.* **269**, 272-282.
Requena, J. (2001). *Proc. Nat. Acad. Sci., U.S.A.* **98**, 69-74.



Ordering Information

| Product # | Description | Pkg. Size |
|-----------|---|------------------|
| 23280 | PeroXOquant™ Quantitative Peroxide Assay Kit <i>Aqueous compatible formulation.</i> Includes: Reagent A (25 mM Ammonium Ferrous Sulfate) Reagent B (125 μM Xylenol Orange in water with Sorbitol) | Kit 2 x 50 ml |
| 23285 | PeroXOquant™ Quantitative Peroxide Assay Kit <i>Lipid-compatible formulation.</i> Includes: Reagent A (25 mM Ammonium Ferrous Sulfate) Reagent B (125 μM Xylenol Orange in methanol with BHT) | Kit 4 x 25 ml |

Comparison of Assay Protocols for Lipid Peroxide Content

PeroXOquant™ Quantitative Peroxidase Assay

1. Mix one volume of Reagent A with 100 volumes of Reagent C to prepare Working Reagent.
2. Add 950 μl of Working Reagent to 50 μl of sample.
3. Incubate at room temperature for 30 minutes.
4. Read at 560 nm (or 595 nm for ELISA plate readers).

Total Time: 35 Minutes

Thiobarbituric Acid Assay

1. Mix 0.1 ml sample, 0.4 ml H_2O and 0.2 ml 7% SDS.
2. Stir gently and add 2 ml 0.1 N HCl.
3. Add 0.3 ml 10% phosphotungstic acid.
4. Incubate 5 minutes at room temperature.
5. Add 1 ml 0.67% thiobarbituric acid (TBA) and acetic acid.
6. Heat 45 minutes at 95°C.
7. Cool in ice bath.
8. Add 5 ml butanol.
9. Vortex and centrifuge for 15 minutes.
10. Determine lipid peroxide concentration in butonal layer by fluorescence at 515 nm excitation and 553 nm emission.

Total Time: 80-90 Minutes