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## Pipetting Tips

One of the most critical aspects of reproducible ELISA assays is to consistently deliver the same amount of liquid with a pipette.

### Calibration

To insure proper calibration, pipette 10 replicates of water at the minimum volume of the pipette into a weigh boat. The CV of the replicates should be less than 2-3%. Repeat with 10 volumes at the maximum volume of the pipette. The CV should be less than 2-3%. If the CV is above 2-3% the pipette needs repair.

### Pipetting Method

To reduce error due to surface tension of the liquid, the following method is recommended. Set the desired volume and pre-rinse the tip with liquid to be pipetted. Depress the plunger to the second stop. Draw in liquid slowly, allowing the plunger to return to the top. Let the liquid reach volumetric equilibrium. Dispense liquid to the first stop. Hold plunger at this stop until pipette is removed from the liquid. Slide the tip on the side of the well to remove any liquid held on the outside of the tip.



### Pipetting Technique

- Maintain consistent speed while pipetting. Avoid sudden motions.
- Insure that the tip is firmly seated on the pipette.
- Change tips between each reagent.
- Use pipette within the range suggested by the manufacturer.
- Pre-rinse the tip with the reagent to be pipetted.
- If the reagent is viscous, pipette slowly and wait until the volume has reached equilibrium before removing the tip from the liquid.
- After drawing up liquid wipe tip with a lint-free tissue
- If an air bubble appears while pipetting, return liquid to the reservoir and re-pipette. If an air bubble continues to appear, replace tip.
- Use separate reservoirs for each reagent

## Making Dilutions

It would seem tempting to make dilutions in a microwell plate using a multichannel pipettor by adding diluent to wells then adding reactant to a row, pipetting up and down a few times and transferring diluted reactant to the next row. There are several reasons not to do this. Pipetting up and down can cause foam, leading to inaccurate amounts being drawn up and an inaccurate dilution (sometimes only in one row of the plate). Secondly, in the time it takes to perform a mixing step either antigen-antibody interactions or adsorption to the plastic will take place, removing some of the reactant from the solution. Instead make all dilutions in low adsorbing test tubes (polypropylene or glass). Add each dilution to a reagent reservoir and add to the plate from the reservoir.

Avoid making large single step dilutions or dilutions which require measurement of a very low volume of reactant. If the dilution is more than 1/1,000, use two steps. For example: make a 1/100 dilution followed by a 1/10 dilution of the 1/100 diluted material. If the amount of reactant needed to make a dilution is 2  $\mu$ l or less, prepare a larger amount than is needed in order to use a larger volume of reactant. This process will be more accurate.



### Reminder:

To make 1.0 ml of a 1/100 dilution. Add 1 $\mu$ l of reagent to 99 $\mu$ l of diluent (not 1 $\mu$ l to 100 $\mu$ l). Better yet, add 5 $\mu$ l of reagent to 495 $\mu$ l of diluent.

## Temperature

A critical factor for both the reactivity and the reproducibility of an ELISA is the temperature at which it is carried out. Most often reactions are carried out at room temperature, but the real source for error exists in not having all the wells come to the same temperature. As mentioned several times, polystyrene is a notoriously poor conductor of heat. The temperature of the reactants inside a well will reflect more closely the temperature which they had going into the well than the temperature of the room. If the temperature of the reactants was 4° C when put into the well, it can be 20 - 30 minutes before they come to room temperature. The step at which this is most critical is the substrate addition step. A "rule of thumb" for enzymes is that a 10° C change in



*Insure that all components come to the temperature at which the reaction will be carried out before adding them to a plate.*

temperature will result in a 2-fold change in activity. While the reaction mixture is not likely to have a 10° C well-to-well difference in temperature, even a 1° C temperature difference is noticeable.

## Timing/Mixing

If reagents are not mixed while in the well, the rate of the reaction is diffusion-dependent. Even in an aqueous system of low viscosity it has been shown that it takes 3 - 4 hours for a binding step to approach equilibrium. If the medium is more viscous (e.g. serum), the rate will be even slower. Typically incubation times for the binding steps of 1 - 2 hours are recommended. Thus it is clearly not the case that they are at equilibrium. One drawback is the lowering of the sensitivity that occurs if binding equilibrium is not reached. However, a compromise between the level of sensitivity needed and the time constraints of completing the assay can usually be achieved. Since binding equilibrium is not likely to have been reached, the most important factor is to be consistent in the timing of the assay from day to day.

Mixing of the plate will speed up the time to reach the binding equilibrium. Vigorous mixing can reduce the required time from 3 - 4 hours to 1 - 2 hours. The key is to mix the assay the same from day to day.

The timing of the substrate conversion step is different. If one is using horseradish peroxidase (HRP), the rate of reaction will most likely slow after 20 minutes if the substrate is TMB. If ABTS is used, a longer incubation time may be possible. If one is using alkaline phosphatase (AP), reaction rates are slower than HRP, but will continue linearly for hours. HRP reaction rates slow due to irreversible substrate inhibition of the enzyme. One can follow the color change and stop the reaction when it



## Critical Factors

### Temperature

- Keep the temperature the same each time the assay is performed
- If the heat has been off over the weekend the lab bench may not be at "room temperature"
- Insure that all components are at the proper temperature before adding them to the assay
- If the assay is to be heated use a heating block rather than an air flow incubator

### Timing/Mixing

- Determine the amount of time and/or mixing needed to attain the needed sensitivity and do it the same each time.

reaches an intensity that is still within the range of readability of the plate reader (O.D. 1.0 - 2.0). When TMB is stopped, it changes color from blue to yellow. Color development increases in intensity approximately 2 - 3 fold and should be stopped when the O.D. reaches 0.7 - 0.9.

## Standard Deviation and Coefficient of Variation

An issue that must be addressed in determining the value of the data generated is the variation of replicate determinations of the same concentration of analyte. An estimate of this is needed both to determine the precision of data points and the minimum amount of analyte that can be detected reproducibly above a determination of zero analyte.

In order to apply these estimates we must assume that the distribution of the values of replicate data points is normal or symmetrical (i.e. there is no skew) around the mean of the values. This may not always be the case and one should determine if the test conditions are causing a skew in the data. To simplify calculations the distribution is often assumed to be normal.



Note - If the values of the mean, the median and the mode of a set of data are identical or very similar, the data is normally distributed.

The standard deviation (SD) provides an estimate of the reproducibility of replicate data points and can provide confidence levels for assessing if one value is truly different from another. Whatever the measured value, a certain percentage of the values obtained are contained within the standard deviation. For instance, one SD on either side of the mean contains 68% of the values under the curve of that distribution. Approximately two SD (actually 1.96 SD) on either side of the mean contains 95% of all of the values and approximately three SD (actually 2.58 SD) contains 99% of all values. Thus if a value that is greater than 3SD different from the mean of a set of samples is obtained, one can be 99% confident that it is truly different from the first set of samples.

Mathematically, the SD is the square root of the sum of the variances squared divided by the number of samples minus one.

$$SD = \sqrt{\frac{(X-x_1)^2 + (X-x_2)^2 + \dots + (X-x_n)^2}{n-1}}$$

The Coefficient of Variation (CV) expresses the SD as a percentage of the mean.

$$\%CV = \frac{SD}{\text{mean}} \times 100$$

## Limit of Detection

The lowest detectable analyte concentration that gives a response which has a statistically significant difference from the response of the zero analyte concentration is the detection limit. In order to have a confidence level of 95%, the means of the replicates of the zero analyte and the unknown concentration must differ by 2 SD and by 3 SD to have a 99% confidence level in the difference.

The factors that determine the ultimate sensitivity of a competitive assay are the antibody affinity constant and the experimental errors but not typically the detectability of the substrate. It has been calculated theoretically that with a  $K = 10^{12} \text{ M}^{-1}$  (an extraordinarily high constant for an antigen-antibody interaction) and a 1% CV for the response at zero dose, the lowest detection limit possible would be  $10^{-14} \text{ M}$ .

The factors limiting the sensitivity of a sandwich assay are the affinity of the antibody, the experimental error and the nonspecific binding of the labeled antibody, expressed as a percentage of the total antibody. It has been estimated that with a  $K = 10^{12} \text{ M}^{-1}$ , 1% CV of the response at zero dose, and a 1% nonspecific binding of the labeled antibody, the detection limit can be as low as  $10^{-16} \text{ M}$ . In addition, this can be enhanced further by using detection substrates with higher detectability.

## Plotting the Data

The ELISA titration data that are generated when increasing concentrations of labeled analyte (or antibody) have been added are typically plotted either linear-linear; log-linear; log-log; or log-logit as illustrated in Figure 15.

### Plots of Immunoassay Data

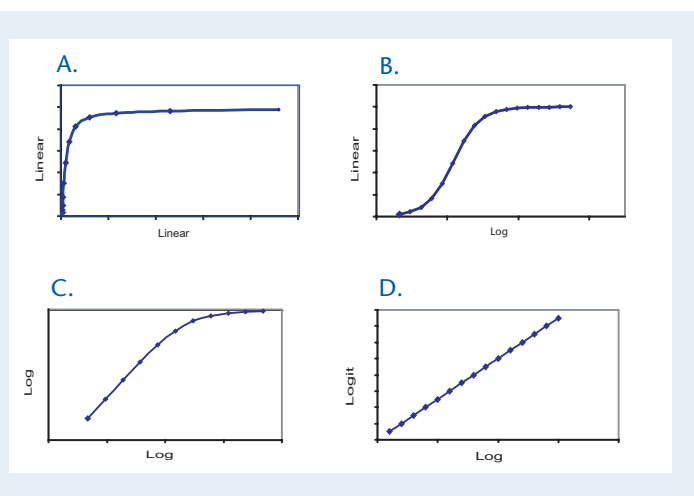


Figure 15.

A linear plot Figure 15A tends to compress the data points that are derived from the lowest concentrations where the interactions are least affected by steric hindrance, competition, or inhibition (the area of best precision). A log-linear (semilog) plot as illustrated in Figure 15B partially overcomes this effect and spreads the data into the typical sigmoid-shaped curve. This plot is often used to compare titration of two samples of labeled component. A more useful plot is illustrated in Figure 15C which shows a log-log plot of the data. In this case the region of receptor excess is completely linearized and ideally will have a slope of one. The linear region will allow a simple curve-fit by linear regression. The final plot (Figure 15D) is a log-logit plot which linearizes both the region of receptor excess and the region of saturation. A log-logit plot requires a highly precise estimate of the signal at saturation. An overestimate will not completely straighten the curve and an underestimate will result in an upward curving plot.

The most useful plot of the data is usually the log-log plot. It provides the most precise estimate of true values in the unsaturated region of the curve. It is easy to fit the data to a curve by linear regression. Deviations of the curve from the ideal are easy to discern and interpret.

Problem	Possible Cause	Solution
<b>High Background</b>	Insufficient Washing	<ul style="list-style-type: none"> <li>• See washing procedure - page 14.</li> <li>• Add detergent to wash solution.</li> <li>• Increase number of washes.</li> <li>• Add 5 minute soak step between washes.</li> <li>• Add protein to the wash solution.</li> </ul>
	Enzyme conjugate at too high a concentration	<ul style="list-style-type: none"> <li>• Check dilution. Titrate if necessary.</li> </ul>
	Insufficient Blocking	<ul style="list-style-type: none"> <li>• Increase blocking protein concentration.</li> <li>• Try a different blocking protein.</li> <li>• Increase blocking time.</li> </ul>
	Incubation times too long	<ul style="list-style-type: none"> <li>• Reduce incubation time.</li> </ul>
	Interfering substance in samples or standards.	<ul style="list-style-type: none"> <li>• Run appropriate controls.</li> <li>• Perform “recovery assay” to determine masking effects.</li> </ul>
	Contaminated buffers	<ul style="list-style-type: none"> <li>• Make fresh buffers.</li> </ul>
<b>No Signal</b>	Reagents added in incorrect order or incorrectly prepared	<ul style="list-style-type: none"> <li>• Repeat assay.</li> <li>• Check calculations and make new buffers, standards, etc.</li> </ul>
	Contamination of enzyme with inhibitor (azide for HRP or phosphate for AP)	<ul style="list-style-type: none"> <li>• Use fresh reagents.</li> </ul>
	Not enough reporter antibody used	<ul style="list-style-type: none"> <li>• Increase concentration.</li> </ul>
	Problems with the standard	<ul style="list-style-type: none"> <li>• Check that standard was handled according to directions.</li> <li>• Use new sample.</li> </ul>
	Capture antibody or analyte did not bind to plate	<ul style="list-style-type: none"> <li>• Restandardize coating conditions.</li> <li>• Increase concentration of coating component.</li> <li>• Increase coating time.</li> <li>• Dilute antibody/analyte in phosphate buffer to insure that no other protein is present.</li> <li>• Change plate type to high binding.</li> <li>• Try covalent linkage plates.</li> </ul>
		Buffers contaminated

7. I'm having trouble. Now what?

Problem	Possible Cause	Solution
<b>Too much signal - plate uniformly reactive</b>	Insufficient washing	<ul style="list-style-type: none"> <li>• See washing procedure - page 14.</li> </ul>
	Substrate solution changed color before use	<ul style="list-style-type: none"> <li>• Use fresh substrate.</li> </ul>
	Too much enzyme conjugate	<ul style="list-style-type: none"> <li>• Check dilution. Titrate if necessary.</li> </ul>
	Plate sealers or reagent reservoirs contaminated	<ul style="list-style-type: none"> <li>• Use only fresh plate sealer and reservoirs.</li> </ul>
	Buffers contaminated	<ul style="list-style-type: none"> <li>• Make fresh.</li> </ul>
<b>Standard curve achieved but poor discrimination between points (low or flat slope)</b>	Not enough enzyme conjugate	<ul style="list-style-type: none"> <li>• Check dilution. Retitrate if necessary.</li> </ul>
	Capture antibody did not bind well to plate	<ul style="list-style-type: none"> <li>• Test different plate types.</li> <li>• Dilute capture antibody in phosphate buffer and insure that no other protein is present.</li> </ul>
	Not enough detection antibody	<ul style="list-style-type: none"> <li>• Check dilution. Retitrate if necessary.</li> </ul>
	Plate not developed long enough	<ul style="list-style-type: none"> <li>• Increase substrate incubation time.</li> </ul>
	Incorrect procedure	<ul style="list-style-type: none"> <li>• Go back to general protocol. Eliminate modifications.</li> </ul>
	Improper calculation of standard curve dilutions	<ul style="list-style-type: none"> <li>• Check calculations and make new standard curve.</li> </ul>
<b>Poor Duplicates</b>	Insufficient washing	<ul style="list-style-type: none"> <li>• See washing procedures on page 14.</li> <li>• If using an automatic plate washer, check that all ports are open and free of obstructions.</li> <li>• Add soak step - see page 15.</li> </ul>
	Uneven plate coating due to procedural error or poor plate quality	<ul style="list-style-type: none"> <li>• Dilute in phosphate buffer without additional protein.</li> <li>• Test coating buffers at different pH.</li> <li>• Check coating and blocking volumes, times and method of reagent addition.</li> <li>• Extend coating time to overnight.</li> <li>• Extend blocking time.</li> <li>• Use certified ELISA plates.</li> </ul>
	Plate sealer reused or no plate sealer used	<ul style="list-style-type: none"> <li>• Use new plates sealers each time.</li> </ul>
	Buffers contaminated	<ul style="list-style-type: none"> <li>• Make fresh buffers.</li> </ul>

Problem	Possible Cause	Solution
<b>Poor assay-to-assay reproducibility</b>	Insufficient washing	<ul style="list-style-type: none"> <li>• See washing procedure - page 14.</li> <li>• If using an automatic plate washer, check that all ports are open and free of obstruction.</li> </ul>
	Variations in incubation temperature	<ul style="list-style-type: none"> <li>• Bring all components to incubation temperature before adding to the wells.</li> <li>• Insure even heating of the plate-polystyrene is a poor conductor of heat.</li> </ul>
	Variations in protocol	<ul style="list-style-type: none"> <li>• Insure standard protocol is followed.</li> </ul>
	Plate sealer reused	<ul style="list-style-type: none"> <li>• Use fresh plate sealer for each step.</li> </ul>
	Improper calculation of standard curve	<ul style="list-style-type: none"> <li>• Recheck calculations.</li> <li>• Make new standard curve.</li> <li>• Use internal controls.</li> </ul>
<b>No signal where expected, but standard curve is fine</b>	No analyte in sample or present at a concentration below the detection limit	<ul style="list-style-type: none"> <li>• Recalibrate amount of sample to use.</li> </ul>
	Sample matrix is masking detection	<ul style="list-style-type: none"> <li>• Perform “recovery assay” to determine masking effects.</li> </ul>
<b>Samples reading above plate reader’s ability to discriminate</b>	Analyte concentration too high	<ul style="list-style-type: none"> <li>• Dilute sample and rerun.</li> </ul>
	Coating concentration too high	<ul style="list-style-type: none"> <li>• Re-develop assay using the same concentration dilution factor for samples and coating solution.</li> </ul>
<b>Low reading across the entire plate</b>	Incorrect wavelength on plate reader	<ul style="list-style-type: none"> <li>• Check maximum absorbance range for the substrate being used.</li> <li>• Check filters.</li> </ul>
	Insufficient development time	<ul style="list-style-type: none"> <li>• Increase development time until background becomes detectable.</li> </ul>
	Stored coated plates are inactive	<ul style="list-style-type: none"> <li>• Coat new plates.</li> <li>• Treat with sucrose before drying.</li> </ul>
	Coated component did not bind well to plate or at too low a concentration	<ul style="list-style-type: none"> <li>• Re-titrate coating conditions.</li> </ul>

**Problem**

**Possible Cause**

**Solution**

**Edge Effects**

Uneven temperature across plate

- Avoid incubating plates in areas where temperature fluctuations may occur.
- Use plate sealers.

## Microwell Plates

Corning - [www.corning.com](http://www.corning.com) - click on "Products and Services" then click on "Life Sciences". This site has a lot of information on plates and aspects of adsorption to solid surfaces.

Nunc - [www.nalgenunc.com](http://www.nalgenunc.com) - click on "Products" then click on "Nunc Brand". This site provides information on plate coating. In addition, search for "covalink" to find information on covalent attachment of molecules to microwells.

The above plates are also easily available through the following suppliers:

VWR - [www.vwrsp.com](http://www.vwrsp.com) search "microwell plates"

Fisher Scientific - [www.fishersci.com](http://www.fishersci.com) search "microwell plates"

## Antibodies

Linscott's directory at [www.linscottsdirectory.com](http://www.linscottsdirectory.com) provides online information on sources of antibodies. Access is available for as little as \$10.00.

**Books** - to order visit [www.amazon.com](http://www.amazon.com)

### ELISA

*Immunochemistry of Solid-Phase Immunoassay* - ed. John E. Butler; CRC Press, 1991.

*Immunoassay* - ed. Eleftherios Diamandis and Theodore Christopoulos; Academic Press; 1996.

### Antibodies

*Using Antibodies - The Sequel to Antibodies, A Laboratory Manual* - Ed Harlow and David Lane; Cold Spring Harbor Press; 1999.

### Conjugating proteins

*Bioconjugate Techniques* - Greg Hermanson; Academic Press; 1996.

## Web Sites

ELISA Assay - [www.biology.arizona.edu](http://www.biology.arizona.edu). Under "Activities" click on "Immunology" or "ELISA". This site illustrates an ELISA assay and describes what it measures and pitfalls in assays.

ELISA Assay - [www.hhmi.org](http://www.hhmi.org). Under "HHMI on the Web" click on "Biointeractive". On the "Biointeractive" page click on "Immunology" and "Virtual Labs". This site offers a virtual tour of an ELISA experiment.

The Centers for Disease Control (CDC) web site has a lot of information and many papers describing ELISA assays. Go to the CDC home page [www.cdc.gov](http://www.cdc.gov) and use the search engine with the term ELISA or EID (Emerging Infectious Diseases - a journal with open access to many papers featuring ELISA). There is also free downloadable Windows-based software for plotting ELISA data.

The following article is recommended as a starting point in developing an ELISA:

Quinn CP, et al. Specific, sensitive, and quantitative enzyme-linked immunosorbent assay for human immunoglobulin G antibodies to anthrax toxin-protective antigen. *Emerg Infect Dis*, Vol 8, No 10, Oct 2002, Available from: URL: <http://www.cdc.gov/ncidod/EID/vol8no10/02-0380.htm>

<b>affinity</b>	The intrinsic attractiveness of one compound for another or the likelihood of staying together once having randomly come together. Between one binding site of an antibody and an epitope it is the three dimensional complementarity; hydrophobic; ionic, vanderWaals and hydrogen bonding forces that control the likelihood of staying together.
<b>affinity purification</b>	A chromatographic purification step in which antibodies are passed over an antigen or epitope attached to a solid surface such as agarose. The antibodies specifically binding to the solid phase antigen can be recovered.
<b>analyte</b>	The antigen, epitope or antibody to be measured by an ELISA.
<b>antibody</b>	A four chain polypeptide that has affinity for a specific epitope. See - " <a href="#">The Use of Antibodies in Immunoassays</a> "
<b>antigen</b>	A molecule that can be recognized by antibodies. Large protein antigens often bear many epitopes; each one is usually distinct. Large carbohydrates, often made up of repeating identical subunits, will typically have repeating epitopes.
<b>anti Ig</b>	Anti-immunoglobulin. An antibody that has affinity for an epitope found on an immunoglobulin molecule. For instance, goats immunized with mouse antibodies will make "goat anti mouse immunoglobulin". See - " <a href="#">The Use of Antibodies in Immunoassays</a> "
<b>ascities</b>	Fluid in the peritoneal cavity. To make ascities containing monoclonal antibody, mice are injected with an irritant to induce fluid and with hybridoma cells secreting a monoclonal antibody through the peritoneum.
<b>avidity</b>	Properties of an antibody other than those defined as affinity that hold an antigen and antibody together and may be defined as the stability of the antibody-antigen complex. IgG and IgA have two binding sites per molecule and IgM has ten. An antigen such as a microbe may have multiple identical epitopes on its surface.
<b>bound</b>	After a period of incubation some portion of the antigen, bearing reactive epitopes, will form a stable complex with one or more antibodies. Those antigens and antibodies which have formed a stable complex are referred to as the bound fraction.
<b>capture antibody</b>	An antibody immobilized on a solid surface used to capture an epitope of interest from the test sample.
<b>carrier</b>	A protein, to which a hapten can be attached, that will render the hapten capable of inducing an immune response.
<b>coefficient of variation (CV)</b>	For normal (Gaussian) distributions, the coefficient of variation measures the relative scatter in data with respect to the mean. It is given as a percentage and is used to compare the consistency or variability of two more series. The higher the C V , the higher the variability, and lower the C. V., the higher the consistency of the data.
<b>critical micelle concentration</b>	The concentration of a detergent above which it forms a micelle rather than being uniformly dispersed.

<b>cross-reaction</b>	The observation that an antibody specific for one antigen may also react with a different antigen. This may occur when the two antigens share a common epitope or epitopes with similar three dimensional shapes so that the antibody can bind either one. Within populations of polyclonal antibodies cross-reactivity may also reflect the fact that one population reacts with one epitope while another population is specific for a different epitope.
<b>ELISA</b>	Enzyme Linked Immunosorbant Assay. ELISA denotes a heterogeneous enzyme-based immunoassay in which one component is attached to a solid surface and enzyme-labeled antibody becomes bound through an epitope-antibody interaction. Unbound component is washed away before adding substrate to measure the amount of enzyme.
<b>epitope</b>	A three-dimensional structure on the solvent-exposed portion of a molecule that interacts with an antibody-binding site.
<b>equilibrium constant</b>	The equilibrium constant or $K$ of an antigen - antibody interaction is the ratio of the on rate ( $k_a$ ) to the off rate ( $k_d$ ). The on rate is controlled by the concentration and mobility of the reactants (dependent on viscosity of the solution, size of the molecules, and whether the reactants are free in solution or restricted in mobility by adsorption to a solid surface). The off rate is controlled by the concentration and stability of the antigen - antibody complex (strength of the hydrophobic interactions, van der Waals forces, hydrogen bonding and ionic interactions that are holding the epitope in the antibody binding site). $K = k_a/k_d$ or $K = [Ag-Ab]/ [Ag] [Ab]$ .
<b>F(ab)</b>	A fragment of an antibody molecule containing a light chain and a VH and a CH1. It has one epitope-binding site.
<b>F(ab')<sub>2</sub></b>	A F(ab) in which the CH1 has been extended to the hinge region and includes S-S bonds holding two F(ab) fragments together. It has two epitope binding sites.
<b>Fc</b>	The C-terminal end of an antibody molecule containing the CH2 and CH3 domains. It does not contain any epitope-binding sites
<b>free</b>	After a period of incubation some portion of the antigen bearing reactive epitopes will be in a stable complex with one or more antibodies. Those antigens which are NOT in a stable complex are referred to as the free fraction.
<b>hapten</b>	A molecule, typically <1000 Dalton, that can be bound by an epitope-binding site, but cannot by itself induce an immune response. It is typically attached to a carrier to induce an immune response.
<b>heterogeneous</b>	An assay in which the free component is washed away from the bound before making a reading.
<b>homogeneous</b>	An assay in which the free and bound enzyme-conjugates do not need to be separated before making a reading. Typically some aspect of the binding step renders the bound enzyme-conjugate active while free enzyme-conjugate remains inactive.
<b>hook effect</b>	A decrease in signal at high doses of analyte when more analyte is added to the assay. Also known as prozone.
<b>hydrophillic</b>	Water-loving. Molecules that are hydrophillic go easily into solution in aqueous buffers.

<b>hydrophobic</b>	Water-hating. Molecules that are hydrophobic are not easily dissolved in aqueous buffers and may require detergents or organic buffers to assist in dissolving them.
<b>immunogen</b>	A molecule which, when injected into an animal, will induce an immune response.
<b>monoclonal antibody</b>	Antibodies derived from one clone of cells. They will have the same binding site. Monoclonal antibodies are obtained by fusing an antibody-producing B cell with a cell line that has infinite ability to divide, then selecting a clone that produces the desired antibody. The resulting fused "hybridoma" cells can secrete the antibody derived from the B cell and have the ability to divide forever.
<b>nitrocellulose (NC)</b>	A popular membrane used as the solid phase in Western blotting. It is a polymer of cellulose in which the --OH group has been modified to --ONO <sub>2</sub> .
<b>parallelism</b>	When the titration curve of the test and sample produce parallel lines.
<b>pI</b>	Isoelectric point - The pH at which the net electric charge on a molecule is zero. On proteins the charge is due to NH <sub>2</sub> → NH <sub>3</sub> <sup>+</sup> and COOH → COO <sup>-</sup> .
<b>polyclonal</b>	Each cell within a clone of B cells secretes identical antibody. When an antigen with multiple epitopes is injected, it is likely that several different clones of B cells will become activated to secrete. The resulting antiserum is referred to as polyclonal.
<b>Protein A/G</b>	Protein A is a cell wall constituent of <i>Staphylococcus aureus</i> ; while Protein G is derived from the cell wall of group G <i>Streptococcus</i> . Both have the ability to bind with high affinity to the Fc region of IgG of numerous mammalian species.
<b>precision</b>	The agreement of replicate measurements. It is a measure of reproducibility but not of the accuracy of the results.
<b>polyvinylidene fluoride (PVDF)</b>	A popular membrane used as the solid phase in Western blotting. It is a polymer of (CH <sub>2</sub> -C[F <sub>2</sub> ]) <sub>n</sub> . In contrast to nitrocellulose (NC), PVDF must be wetted by alcohol before use. It has a higher binding capacity than NC.
<b>sensitivity</b>	The minimal detectable amount of an analyte that can be distinguished reproducibly from zero analyte. This is often referred to as the limit of detection.
<b>standard curve</b>	A curve or straight line produced by mathematically fitting a curve to the data derived from a reference or known standard.
<b>standard deviation</b>	The average amount by which data points deviate from the mean. It is the square root of the variance.
<b>Western blot</b>	A solid phase immunoassay in which proteins are transferred from a polyacrylamide gel after electrophoresis to a membrane such as nitrocellulose or PVDF. After transfer detection of the protein bands can be accomplished using antibody-enzyme conjugates.

## Related Products:

Protein Detector ELISA kits provide a convenient starting point for the development of ELISA protocols. Each kit includes a comprehensive instruction manual for developing enzyme immunoassay procedures as well as pretested components including blocking, coating and wash solutions along with affinity-purified conjugates.

<a href="#">HRP ELISA Kits</a>	Catalog No.	Size
Protein Detector™ ELISA Kit, HRP System - Anti Human IgG (H+L)	54-62-10	2000 tests
Protein Detector ELISA Kit, HRP System - Anti-Mouse IgG (H+L)	54-62-18	2000 tests
Protein Detector ELISA Kit, HRP System Anti-Rabbit IgG (H+L)	54-62-15	2000 tests

<a href="#">AP ELISA Kits</a>	Catalog No.	Size
Protein Detector ELISA Kit, AP System - Anti-Mouse & Anti-Rabbit IgG (H+L)	55-81-50	2000 tests
Protein Detector ELISA Kit, AP System - Anti-Human IgG (H+L)	55-81-10	2000 tests

<a href="#">Alkaline Phosphatase (AP) -labeled Antibodies</a>	Catalog No.	Size
<a href="#">Anti-Human</a>		
AP-labeled Goat Anti-Human IgG (H+L)	05-10-06	0.1 mg
AP-labeled Goat Anti-Human IgG(Fc)	05-10-20	0.1mg
AP-labeled Goat Anti-Human IgA (α)	075-1001	1.0 mg
AP-labeled Goat Anti-Human IgG (γ)	075-1002	1.0 mg
AP-labeled Goat Anti-Human IgM (μ)	075-1003	1.0 mg
AP-labeled Goat Anti-Human IgE (ε)	075-1004	1.0 mg
AP-labeled Goat Anti-Human IgG (H+L)	075-1006	1.0 mg
AP-labeled Goat Anti-Human IgA + IgG + IgM (H+L)	075-1007	1.0 mg
AP-labeled Goat Anti-Human IgG (γ), liquid	475-1002	1.0 mL
AP-labeled Goat Anti-Human IgM (μ), liquid	475-1003	1.0 mL
AP-labeled Goat Anti-Human IgG (H+L), liquid	475-1006	1.0 mL
<a href="#">Anti-Mouse</a>		
AP-labeled Goat Anti-Mouse IgG (H+L), HSA	05-18-06	0.1 mg
AP-labeled Goat Anti-Mouse IgG( H+L), RtSA+HSA	05-18-15	0.1 mg
AP-labeled Goat Anti-Mouse IgG (H+L), RbSA+HSA	05-18-18	0.1 mg
AP-labeled Goat Anti-Mouse IgA (α), HSA	15-18-01	0.5 mg
AP-labeled Goat Anti-Mouse IgG (γ), HSA	075-1802	1.0 mg
AP-labeled Goat Anti-Mouse IgM (μ), HSA	075-1803	1.0 mg
AP-labeled Goat Anti-Mouse IgG (H+L), HSA	075-1806	1.0 mg
AP-labeled Goat Anti-Mouse IgA+IgG+IgM (H+L), HSA	075-1807	1.0 mg
AP-labeled Goat Anti-Mouse IgG+IgM (H+L), HSA	075-1809	1.0 mg
AP-labeled Goat Anti-Mouse IgG (γ), HSA, liquid	475-1802	1.0 mL
AP-labeled Goat Anti-Mouse IgG (H+L), HSA, liquid	475-1806	1.0 mL
AP-labeled Goat Anti- <i>Peromyscus Leucopus</i> IgG (H+L)	15-33-06	0.5 mg
<a href="#">Anti-Rabbit</a>		
AP-labeled Goat Anti-Rabbit IgG (H+L)	075-1506	1.0 mg
AP-labeled Goat Anti-Rabbit IgG (H+L), HSA	075-1516	1.0 mg
AP-labeled Goat Anti-Rabbit IgG (H+L), liquid	475-1506	1.0 mL
AP-labeled Goat Anti-Rabbit IgG (H+L), HSA, liquid	475-1516	1.0 mL
AP-labeled Goat Anti-Rabbit IgG (H+L)	05-15-06	0.1 mg

HSA = Human Serum Adsorbed  
 MSA = Mouse Serum Adsorbed  
 RbSA = Rabbit Serum Adsorbed  
 RtSA = Rat Serum Adsorbed

**Alkaline Phosphatase (AP) -labeled Antibodies (continued)**

**Anti-Rat**

	Catalog No.	Size
AP-labeled Goat Anti-Rat IgG (H+L)	15-16-06	0.5 mg
AP-labeled Goat Anti-Rat IgG (γ)	05-16-02	0.1 mg
AP-labeled Goat Anti-Rat IgM (μ)	05-16-03	0.1 mg
AP-labeled Goat Anti-Rat IgG (H+L), MSA	05-16-02	0.1 mg
AP-labeled Goat Anti-Rat IgG (H+L), MSA, liquid	475-1612	1.0 mL

**Horseradish Peroxidase (HRP) - labeled Antibodies**

**Anti-Human**

	Catalog No.	Size
HRP-labeled Goat Anti-Human IgG (H+L)	04-10-06	0.1 mg
HRP-labeled Goat Anti-Human IgA+IgG+IgM (H+L) MSA	04-10-17	0.1 mg
HRP-labeled Goat Anti-Human IgG (Fc)	04-10-20	0.1 mg
HRP-labeled Goat Anti-Human IgA (α)	14-10-01	0.5 mg
HRP-labeled Goat Anti-Human IgG (γ)	074-1002	1.0 mg
HRP-labeled Goat Anti-Human IgM (μ)	074-1003	1.0 mg
HRP-labeled Goat Anti-Human IgE (ε)	074-1004	1.0 mg
HRP-labeled Goat Anti-Human IgG (H+L)	074-1006	1.0 mg
HRP-labeled Goat Anti-Human IgA+IgG+IgM (H+L)	074-1007	1.0 mg
HRP-labeled Goat Anti-Human IgG (γ), liquid	474-1002	1.0 mL
HRP-labeled Goat Anti-Human IgM (μ), liquid	474-1003	1.0 mL
HRP-labeled Goat Anti-Human IgG (H+L), liquid	474-1006	1.0 mL

**Anti-Mouse**

HRP-labeled Goat Anti-Mouse IgG (H+L), HSA	04-18-06	0.1 mg
HRP-labeled Goat Anti-Mouse IgG (H+L), RtSA + HSA	04-18-15	0.1 mg
HRP-labeled Goat Anti-Mouse IgG (H+L), RbSA + HSA	04-18-18	0.1 mg
HRP-labeled Goat Anti-Mouse IgA (α), HSA	14-18-01	0.5 mg
HRP-labeled Goat Anti-Mouse IgG (γ), HSA	074-1802	1.0 mg
HRP-labeled Goat Anti-Mouse IgM (μ), HSA	074-1803	1.0 mg
HRP-labeled Goat Anti-Mouse (H+L), HSA	074-1806	1.0 mg
HRP-labeled Goat Anti-Mouse IgA+IgG+IgM (H+L), HSA	074-1807	1.0 mg
HRP-labeled Goat Anti-Mouse IgG + IgM (H+L), HSA	074-1809	1.0 mg
HRP-labeled Goat Anti-Mouse IgG (γ), HSA, liquid	474-1802	1.0 mL
HRP-labeled Goat Anti-Mouse IgG (H+L), HSA, liquid	474-1806	1.0 mL
HRP-labeled Goat Anti- <i>Peromyscus Leucopus</i> IgG (H+L)	14-33-06	0.5 mg

**Anti-Rabbit**

HRP-labeled Goat Anti-Rabbit IgG (H+L)	074-1506	1.0 mg
HRP-labeled Goat Anti-Rabbit IgG (H+L)	04-15-06	0.1 mg
HRP-labeled Goat Anti-Rabbit IgG (H+L), HSA	074-1516	1.0 mg
HRP-labeled Goat Anti-Rabbit IgG (H+L), liquid	474-1506	1.0 mL

**Anti-Rat**

HRP-labeled Goat Anti-Rat IgG (γ)	04-16-02	0.1 mg
HRP-labeled Goat Anti-Rat IgM (μ)	04-16-03	0.1 mg
HRP-labeled Goat Anti-Rat IgG (H+L)	14-16-06	0.5 mg
HRP-labeled Goat Anti-Rat IgG (H+L), MSA	14-16-12	0.5 mg
HRP-labeled Goat Anti-Rat IgG (H+L), MSA, liquid	474-1612	1.0 mL

HSA = Human Serum Adsorbed  
 MSA = Mouse Serum Adsorbed  
 RbSA = Rabbit Serum Adsorbed  
 RtSA = Rat Serum Adsorbed

**Biotin- labeled Antibodies**

**Catalog No.**

**Size**

**Anti-Human**

Biotin-labeled Goat Anti-Human IgA (α)	16-10-01	0.5 mg
Biotin-labeled Goat Anti-Human IgG (γ)	16-10-02	0.5 mg
Biotin-labeled Goat Anti-Human IgM (μ)	16-10-03	0.5 mg
Biotin-labeled Goat Anti-Human IgE (ε)	16-10-04	0.5 mg
Biotin-labeled Goat Anti-Human IgG (H+L)	16-10-06	0.5 mg
Biotin-labeled Goat Anti-Human IgA+IgG+IgM (H+L)	16-10-07	0.5 mg
Biotin-labeled Goat Anti-Human IgG (H+L)	176-1006	2.0 mg

**Anti-Mouse**

Biotin-labeled Goat Anti-Mouse IgA (α), HSA	16-18-01	0.5 mg
Biotin-labeled Goat Anti-Mouse IgG (γ), HSA	16-18-02	0.5 mg
Biotin-labeled Goat Anti-Mouse IgM (μ), HSA	16-18-03	0.5 mg
Biotin-labeled Goat Anti-Mouse IgG (H+L), HSA	16-18-06	0.5 mg
Biotin-labeled Goat Anti-Mouse IgA+IgG+IgM (H+L), HSA	16-18-07	0.5 mg
Biotin-labeled Goat Anti-Mouse IgG+IgM (H+L), HSA	16-18-09	0.5 mg
Biotin-labeled Goat Anti-Mouse IgG (H+L), RtSA+HSA	16-18-15	0.5 mg
Biotin-labeled Goat Anti-Mouse IgG (H+L) RbSA+HSA	16-18-18	0.5 mg
Biotin-labeled Goat Anti-Mouse IgG (H+L), HSA	176-1806	2.0 mg

**Anti-Rabbit**

Biotin-labeled Goat Anti-Rabbit IgG (H+L)	16-15-06	0.5 mg
Biotin-labeled Goat Anti-Rabbit IgG (H+L), HSA	16-15-16	0.5 mg
Biotin-labeled Goat Anti-Rabbit IgG (H+L)	176-1506	2.0 mg

**Anti-Rat**

Biotin-labeled Goat Anti-Rat IgG (γ)	16-16-02	0.5 mg
Biotin-labeled Goat Anti-Rat IgM (μ)	16-16-03	0.5 mg
Biotin-labeled Goat Anti-Rat IgG (H+L)	16-16-06	0.5 mg
Biotin-labeled Goat Anti-Rat IgG (H+L), MSA	16-16-12	0.5 mg

**Labeled Streptavidin and Protein A**

**Catalog No.**

**Size**

**Labeled Streptavidin**

HRP-labeled	14-30-00	0.5 mg
HRP-labeled, liquid	474-3000	1.0 mL
AP-labeled, liquid	475-3000	1.0 mL
AP-labeled	15-30-00	0.5 mg

**Labeled Protein A**

HRP-labeled	14-50-00	0.5 mg
FITC-labeled	12-50-00	0.5 mg

HSA = Human Serum Adsorbed  
 MSA = Mouse Serum Adsorbed  
 RbSA = Rabbit Serum Adsorbed  
 RtSA = Rat Serum Adsorbed

**Substrates for ELISA**

**Catalog No.**

**Size**

**Phosphatase Chromogenic Substrates**

BluePhos® Microwell Substrate Kit	50-88-00	6 x 100 mL
BluePhos Microwell Substrate Kit	50-88-01	6 x 450 mL
BluePhos Microwell Substrate Kit	50-88-02	2 x 25 mL
BluePhos Stop Solution (10X Concentrate)	50-89-00	2 x 100 mL
pNPP Microwell Substrate System	50-80-00	500 mL
pNPP Microwell Substrate	50-80-01	100 x 5 mg tabs

**Peroxidase Chromogenic Substrates**

SureBlue™ TMB Microwell Peroxidase Substrate Kit	52-00-01	100 mL
SureBlue TMB Microwell Peroxidase Substrate Kit	52-00-02	4 x 100 mL
SureBlue TMB Microwell Peroxidase Substrate	52-00-03	1 L
SureBlue Reserve™ TMB Microwell Substrate	53-00-01	100 mL
SureBlue Reserve TMB Microwell Substrate	53-00-02	4 x 100 mL
SureBlue Reserve TMB Microwell Substrate	53-00-03	1000 mL
TMB Microwell Peroxidase Substrate Kit	50-76-00	6 x 100 mL
TMB Microwell Peroxidase Substrate Kit	50-76-03	6 x 450 mL
TMB Stop Solution Kit	50-85-05	4 x 100 mL
TMB Stop Solution	50-85-06	1 L
ABTS® Microwell Peroxidase Substrate Kit	50-66-00	6 x 100 mL
ABTS Microwell Peroxidase Substrate	50-66-06	1000 mL
ABTS Microwell Peroxidase Substrate	50-66-18	100 mL
ABTS Microwell Peroxidase Substrate Kit	50-62-00	6 x 100 mL
ABTS Microwell Peroxidase Substrate Kit	50-62-01	6 x 450 mL
ABTS Peroxidase Stop Solution Kit	50-85-01	2 x 100 mL

**ELISA Peroxidase Chemiluminescent Substrates**

**Catalog No.**

**Size**

LumiGLO® Chemiluminescent Substrate Kit	54-61-00	2 x 120 mL
LumiGLO Chemiluminescent Substrate Kit	54-61-01	6 x 120 mL
LumiGLO Chemiluminescent Substrate Kit	54-61-02	2 x 30 mL
LumiGLO Reserve™ Chemiluminescent Substrate Kit	54-71-00	2400 cm <sup>2</sup>
LumiGLO Reserve Chemiluminescent Substrate Kit	54-71-01	600 cm <sup>2</sup>
CDP-Star® Chemiluminescent Substrate	50-60-05	100 mL

**BacTrace Antibodies to Bacteria**

**Catalog No.**

**Size**

**Phosphatase (AP) -labeled**

AP-labeled Goat Anti- <i>Listeria</i> , Genus Specific	05-90-90	0.1 mg
AP-labeled Goat Anti- <i>Salmonella</i> , CSA-1	05-91-99	0.1 mg
AP-labeled Goat Anti- <i>Campylobacter</i> Species	05-92-93	0.1 mg
AP-labeled Goat Anti- <i>Escherichia coli</i> (O157:H7)	05-95-90	0.1 mg
AP-labeled Goat Anti- <i>Renibacterium salmoninarum</i>	05-96-91	0.1 mg
AP-labeled Goat Anti- <i>Borrelia burgdorferi</i>	05-97-91	0.1 mg

**Peroxidase (HRP) - labeled**

HRP-labeled Goat Anti- <i>Listeria</i> , Genus Specific	04-90-90	0.1 mg
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HSA = Human Serum Adsorbed  
 MSA = Mouse Serum Adsorbed  
 RbSA = Rabbit Serum Adsorbed  
 RtSA = Rat Serum Adsorbed

### BacTrace Antibodies to Bacteria (con't)

	Catalog No.	Size
HRP-labeled Goat Anti- <i>Salmonella</i> , CSA-1	04-91-99	0.1 mg
HRP-labeled Goat Anti- <i>Campylobacter</i> Species	04-92-93	0.1 mg
HRP-labeled Goat Anti- <i>Helicobacter pylori</i>	04-93-94	0.1 mg
HRP-labeled Goat Anti- <i>Escherichia coli</i> (O157:H7)	04-95-90	0.1 mg
HRP-labeled Goat Anti- <i>Renibacterium salmoninarum</i>	04-96-91	0.1 mg
HRP-labeled Goat Anti- <i>Borrelia burgdorferi</i>	04-97-91	0.1 mg
HRP-labeled Goat Anti- <i>Borrelia</i> species	04-97-92	0.1 mg

### BacTrace Positive Controls

Positive Control, <i>Salmonella typhimurium</i>	50-74-01	1.0 mL
Positive Control, <i>Listeria</i> , Genus Specific	50-90-90	1.0 mL
Positive Control, <i>Campylobacter jejuni</i> , Genus Specific	50-92-93	1.0 mL
Positive Control, <i>Helicobacter pylori</i>	50-93-94	1.0 mL
Positive Control, <i>Escherichia coli</i> (O157:H7)	50-95-90	1.0 mL
Positive Control, <i>Renibacterium salmoninarum</i>	50-96-91	1.0 mL
Positive Control, Anti- <i>Borrelia burgdorferi</i>	50-97-91	1.0 mL

### Assay Support Reagents and Accessories

	Catalog No.	Size
<u>Immunoassay Support Reagents</u>		
Coating Solution Concentration Kit	50-84-00	2 x 25 mL
HRP Stabilizer	54-15-01	200 mL
AP Stabilizer	55-15-00	200 mL
10% Normal Goat Serum	71-00-27	50 mL
10% Normal Rabbit Serum	71-00-28	50 mL
10% Normal Mouse Serum	71-18-01	10 mL
10% BSA Diluent/Blocking Kit	50-61-00	2 x 100 mL
10% BSA Diluent/Blocking Solution	50-61-10	1 L
Milk Diluent/Blocking Concentration Kit	50-82-01	2 x 100 mL
Detector Block (5X)	71-83-00	240 mL
Wash Solution Concentration Kit	50-63-00	4 x 200 mL
Biotin Wash Kit (10X)	50-63-06	2 x 100 mL
ABTS Peroxidase Stop Solution Kit	50-85-01	2 x 100 mL
TMB Stop Solution	50-85-05	4 x 100 mL
TMB Stop Solution	50-85-06	1 L
BluePhos Stop Solution (10X Concentrate)	50-89-00	2 x 100 mL
DEA Buffer Solution	50-80-02	100 mL
Phosphatase Assay Buffer (10X)	50-63-14	200 mL

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 MSA = Mouse Serum Adsorbed  
 RbSA = Rabbit Serum Adsorbed  
 RtSA = Rat Serum Adsorbed

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