ELISA GUIDE

A CLEAR AND EASY GUIDE TO ELISAS

Reed Biotech

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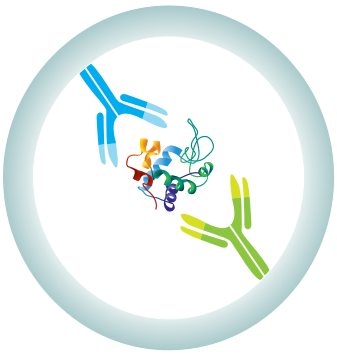
What is an ELISA?



ELISAs (Enzyme Linked Immunosorbent Assays) are a type of immunoassay that are commonly used to quantify levels of a specific target within a sample. Samples routinely used in ELISAs include serum, plasma, cell culture supernates, cell lysates, saliva, tissue lysates, and urine.

ELISAs are usually run in 96-well microplates coated with a capture antibody specific for the analyte of interest. Upon incubation with experimental samples, standards, or controls, the target analyte is captured by this antibody. A conjugated detection antibody binds to a different epitope on the target analyte. A substrate solution is subsequently added to produce a signal that is proportional to the amount of analyte bound. ELISAs can have different formats. Descriptions and diagrams of these can be found in the next section.

The Highest-Quality ELISAs Available

ReeD Biotech®, a New Star Biotechnology brand, Researchers has years of experience designing, testing, and optimizing immunoassay kits to ensure the highest level of performance in analyte quantification. We currently offer more than 1200 complete, ready-to-use Traditional conventions ELISA Kits, 200 Micro Fast ELISA Development Systems for numerous different analytes and species, including human, mouse, rat, canine, primate, and porcine, and an automated ELISA workstation with High quality testing services. Choosing quality reagents that will lead to results you can trust is one of the most critical aspects of scientific research.

- New Generation ELISA Manufacturer
- Flexible Formats available
- Extensive Analyte Selection
- Rigorous Validation Testing
- Extensive Quality Control Testing
- Long-term Consistency
- Bulk Packaging Available

What's at the core of your immunoassay?

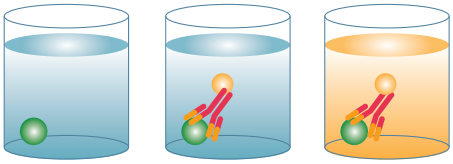
Your results matter, so what's inside your immunoassay should too. ReeD Biotech® antibodies and proteins are the core of every REEDBIO immunoassay platform. Our antibodies and proteins are highly specific, manufactured in-house to ensure reproducibility and tested for suitability on every application we develop. In addition, the proteins used for the immunoassay standard and as antibody immunogens are typically full-length, recombinant proteins that are confirmed to be biologically active. The ensures that our standard closely mimics the natural protein and that the antibodies will recognize the native for of the analyte.

ELISA Types

The four main types of ELISAs are indirect, direct, sandwich, and competitive. Each type of ELISA has its own advantages and disadvantages.

Direct ELISA

In a direct ELISA, an antigen or sample is immobilized directly on the plate and a conjugated detection antibody binds to the target protein. Substrate is then added, producing a signal that is proportional to the amount of analyte in the sample. Since only one antibody is used in a direct ELISA, they are less specific than a sandwich ELISA.



When to Use

Assessing antibody affinity and specificity. Investigating blocking/inhibitory interactions

Advantages

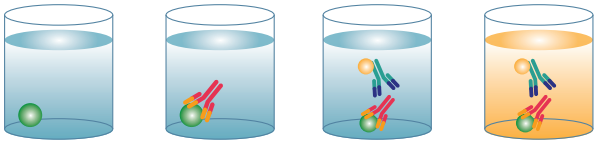
- Fast and simple protocol

Disadvantages

- Less specific since you are only using 1 antibody.
- Potential for high background if all proteins from a sample are immobilized in well.

Indirect ELISA

An indirect ELISA is similar to a direct ELISA in that an antigen is immobilized on a plate, but it includes an additional amplification detection step. First, an unconjugated primary detection antibody is added and binds to the specific antigen. A conjugated secondary antibody directed against the host species of the primary antibody is then added. Substrate then produces a signal proportional to the amount of antigen bound in the well.



When to Use

Measuring endogenous antibodies

Advantages

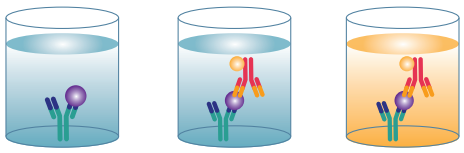
- Amplification using a secondary antibody

Disadvantages

- Potential for cross-reactivity caused by secondary antibody

Sandwich ELISA

Sandwich ELISAs are the most common type of ELISA. Two specific antibodies are used to sandwich the antigen, commonly referred to as matched antibody pairs. Capture antibody is coated on a microplate, sample is added, and the protein of interest binds and is immobilized on the plate. A conjugated-detection antibody is then added and binds to an additional epitope on the target protein. Substrate is added and produces a signal that is proportional to the amount of analyte present in the sample. Sandwich ELISAs are highly specific, since two antibodies are required to bind to the protein of interest.



When to Use

Determining analyte concentration in a biological sample

Advantages

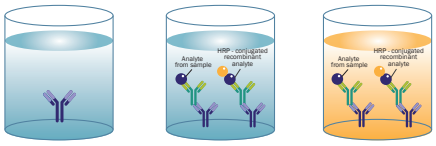
- Highest specificity and sensitivity
- Compatible with complex sample matrices

Disadvantages

- Longer protocol
- Challenging to develop

Competitive ELISA

Competitive ELISAs are commonly used for small molecules, when the protein of interest is too small to efficiently sandwich with two antibodies. Similar to a sandwich ELISA, a capture antibody is coated on a microplate. Instead of using a conjugated detection antibody, a conjugated antigen is used to complete for binding with the antigen present in the sample. The more antigen present in the sample, the less conjugated antigen will bind to the capture antibody. Substrate is added and the signal produced is inversely proportional to the amount of protein present in the sample.



When to Use

Determining concentrations of small molecules and hormones

Advantages

- Ability to quantitate small molecules

Disadvantages

- Less specific since you are only using 1 antibody
- Requires a conjugated antigen

Why use an ELISA over other techniques?

There are many different immunoassay platforms available to measure protein levels in biological fluids. ELISAs are preferred in many cases due to their sensitivity, specificity, accuracy, and ability to tolerate harsh buffers or pretreatments. Comparing an ELISA to a Western blot, sandwich ELISAs use 2 specific antibodies rather than one and allow for completely quantitative results, while a Western blot can see non-specific bands and are semi-quantitative at best. An advantage of ELISAs over different multiplexing platforms is the ability to customize the assay for the target analyte and not having to worry about interference caused by many other antibodies and proteins working together. The diluents used in our Traditional conventions ELISA kits are fully optimized to achieve the best performance for that analyte in complex sample matrices. The potential of observing cross-reactivity or interference is minimized and you can push the sensitivity limits with this technique.

ELISA Formats

Which Immunoassay is Right for You?

KIT	Traditional ELISA	Micro Fast ELISA	High Sensitive ELISA	Universal small molecules	OEM Services
Format	96-well plate	96-well plate	96-well plate	96-well plate	Flexible
Benefit	• Most Choose • Low CVs	• Fastest Plate-based ELISA	• Highest Sensitivity	• Economical • Largest Menu • Flexible	• Hands Free • Sensitive
Sample Volume	100 µL	25 µL	100 µL	50 µL	10-100µL
Number of Analytes	1	1	1	1	1
Assay Time	3.5 Hours	≤ 3 Hours	3 Hours	90 minutes	90 minutes
Pre-coated	Yes	Yes	Yes	Yes	Yes

New Generation ELISA Manufacturer: Reed Biotech® ELISAs

Kit Components

- Pre-coated 96-well Microplate
- Conjugated Detection Antibody
- Calibrated Immunoassay Standard
- Assay Diluent
- Calibrator Diluent(s)
- Wash Buffer
- TMB Substrate Reagent
- Stop Solution
- Plate Sealers

Traditional Colorimetric Sandwich ELISA Kits

Traditional ELISA Kits are complete, ready-to-use kits that represent the gold standard in single analyte detection. Kits are available for measuring a wide range of molecules including cytokines, chemokines, growth factors, proteases, and more.

NEW

MF® Micro Fast Colorimetric Sandwich ELISA Kits

Micro Fast ELISAs allow for rapid quantitation of target protein in only 180 minutes. With a shortened protocol and only two wash step, these ready-to-use kits allow you to accomplish more in your day, while getting the same quality of a Traditional ELISA.

HS® High Sensitivity Colorimetric Sandwich ELISA Kit

HS ELISA Kits are complete assays generally used when very low levels of the target protein are expected. These kits utilize distinct protocols to achieve their impressive levels of analyte detection. Two different amplification systems are available.

Parameter Colorimetric ELISA Kits
Universal small molecules

Parameter ELISA kits are complete microplate-based assays designed to accurately measure the levels of small molecules using the competitive ELISA format.

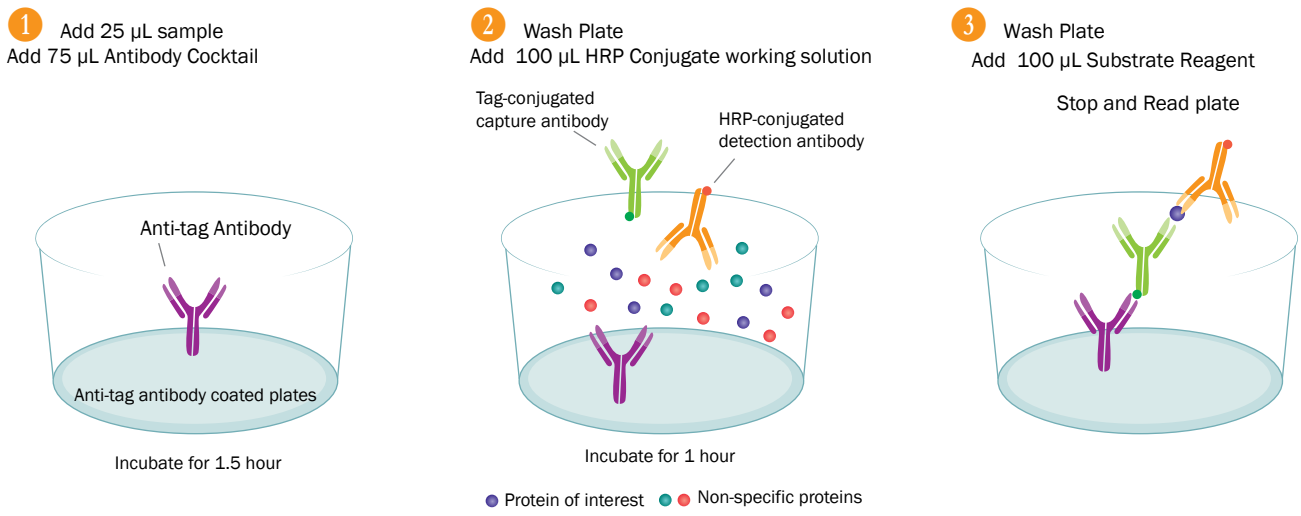
OEM Services

Metabolism Assays use a chemiluminescent substrate for analyte detection and require a luminometer for output reading. These kits have a broad dynamic range in comparison to standard colorimetric immunoassays.

New Micro Fast Colorimetric Sandwich ELISA Kits

Accomplish more in your day without compromising quality. The Micro Fast ELISAs provide quick, accurate quantitation of proteins in serum, plasma, and cell culture supernates. Unlike traditional ELISAs, MF ELISAs have a fast, simplified protocol that only takes 180 minutes to results with just two wash step. You can expect the same high quality results, sensitivity, and lot-to-lot consistenct to our existing gold-standard Micro Fast ELISA kits.

How Micro Fast ELISA Assays Work



Superior Linearity and Recovery

Linearity and recovery are key performance criteria to ensure your assay is accurately detecting samples, identifying false positives, and demonstrating your assay is ideal for your sample matrix. Reed Biotech® ELISA kits excel in this area and the Micro Fast ELISAs are no different.

Add data and legends from linearity and recovery document attached to email.

Get Micro Fast quality in a fraction of the time!

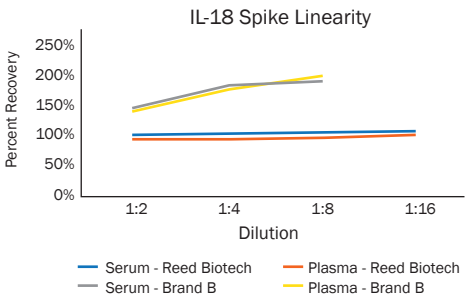


Figure 1. The IL-18 Micro Fast ELISA has superior linearity to Brand B. At dilute concentrations, Brand B is susceptible to matrix effects.

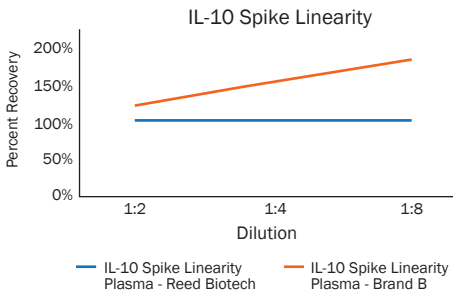


Figure 2. The Reed Biotech® IL-10 Micro Fast™ has superior linearity to the Brand B counterpart.

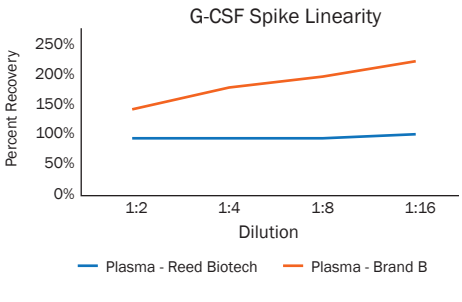
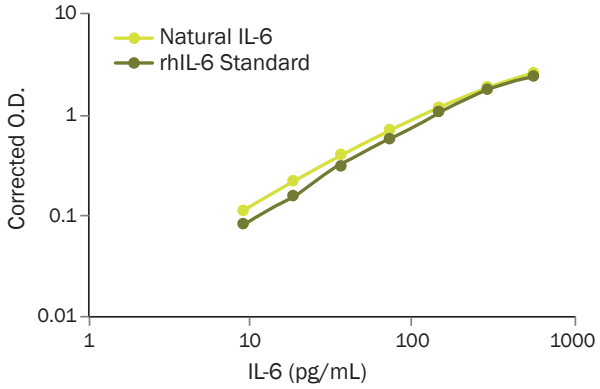


Figure 3. The Reed Biotech® G-CSF Micro Fast™ has superior linearity to the Brand B counterpart.

Ensuring ELISA Performance and Consistency

Accurate Detection of Natural Proteins

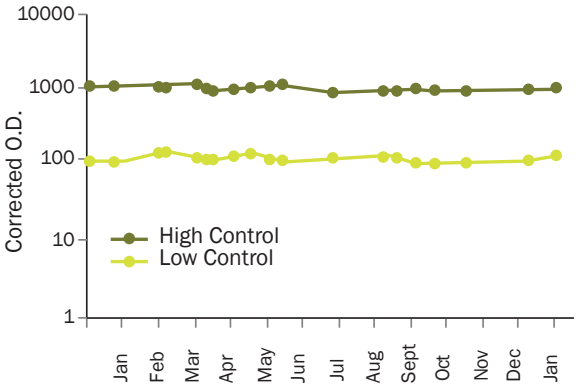
Antibody pairs recognize the supplied recombinant standard and the natural proteins in biological samples in a parallel manner, confirming that this kit can be used to measure the relative mass values of the natural analyte. Reed Biotech has determined the ideal standard curve range for each assay, ensuring peak sensitivity and reproducibility of results.



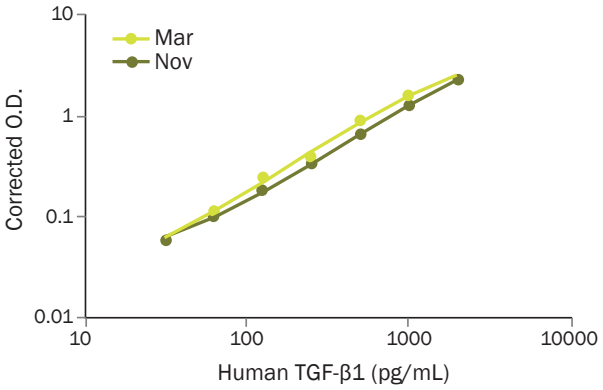
Recognition of Recombinant and Natural Human IL-6. Serial dilutions of rhIL-6 standard (dark green line) or natural IL-6 produced by unstimulated monocytes (light green line) were quantitated using the Human IL-6 Micro Fast ELISA Kit (Catalog # RE3186HF). Micro Fast ELISAs detect both recombinant and natural proteins in a parallel manner across a range of concentrations.

Confirmed Lot-to-Lot Consistency

All lots are tested to ensure low background, a linear standard curve, consistent assay sensitivity, and a broad dynamic standard curve range. Consistent standard curve O.D.s, control values, and natural sample values ensure that your samples run consistently over time.



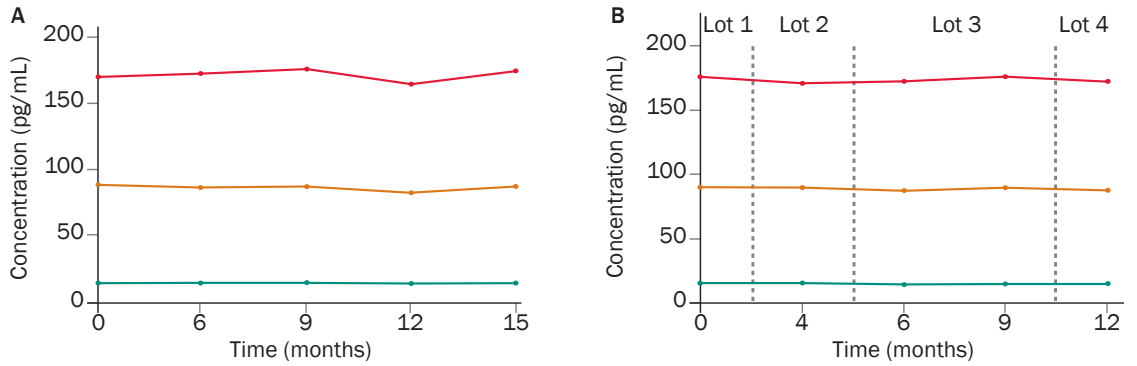
Quantitation of Human TGF-β1 in High and Low Controls. Controls are assayed with every manufactured lot of TGF-β1 Micro Fast ELISA. Controls must read within a set range of ± two standard deviations from the mean. Controls for the TGF-β1 Micro Fast ELISA System have remained consistent across 13 months.



Comparison of Human TGF-β1 Standard Curve O.D.s from 8 months. Using the Human TGF-β1 Micro Fast ELISA System (Catalog # RE10013F), standard curve values generated in Mar and Nov were compared for lot-to-lot consistency. Standard curve O.D.s remained consistent over 8 months.

Precision & Reproducibility: Providing Confidence in Your Results

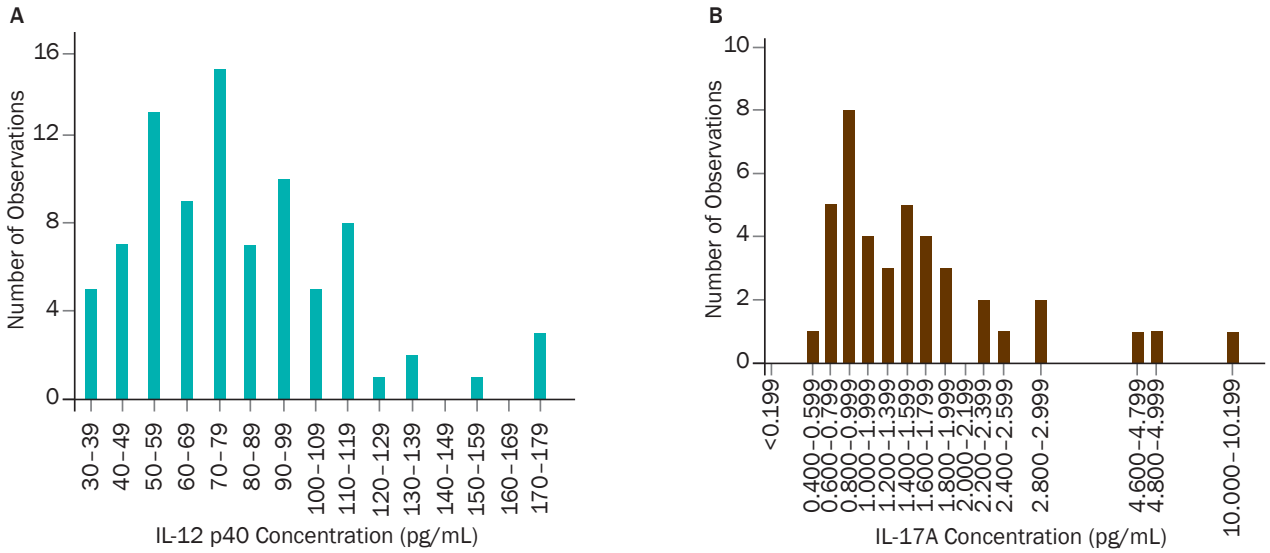
Immunoassay precision is defined as the reproducibility of results within and between assays. This characteristic of an immunoassay is extremely important in order to: 1) provide assurance that the results obtained throughout a study are accurate and reproducible from one experiment to the next and 2) determine if two results are the same or different. Precision is measured as a coefficient of variation (CV) from the mean value. Two types of precision should be considered, intra-assay precision and inter-assay precision. Intra-assay precision is the reproducibility between wells within an assay. This allows the researcher to run multiple replicates of the same sample on one plate and obtain similar results. Inter-assay precision is the reproducibility between assays. Inter-assay precision guarantees that the results obtained will be reproducible using multiple kits over a period of time. Reed Biotech Traditional conventions Immunoassays typically have CV values less than 10% across the standard curve for both intra- and inter-assay precision. These low CV values allow the researcher to perform repeated assays and be confident that the results are consistent throughout the study.



Reed Biotech Traditional conventions ELISA Kits Are Tested for Stability and Reproducibility. A. Three samples with different concentrations of IL-6 (colored lines) were assayed using the same lot of the Human IL-6 ELISA Kit (Catalog # RE3186H) over a 15 month period. **B.** Three samples with differing IL-6 concentrations (colored lines) were assayed using four different lots of the Human IL-6 ELISA Kit (Catalog # RE3186H) over a 12 month period.

Sensitivity: Measuring Proteins at the pg/mL Range

The minimum detectable dose is the lowest measurable value that is statistically different from zero. It is calculated by adding two standard deviations to the mean optical density value of several zero standard replicates and determining the corresponding analyte concentration from the standard curve. The better the sensitivity of an assay, the lower the useful working range (standard curve range) will be. Reed Biotech Traditional conventions ELISAs are optimized to ensure high signal, low background, and the best sensitivity possible.



The Minimum Detectable Dose for Many Reed Biotech ELISA Kits Allows Proteins Present at the pg/mL Range to be Accurately Measured. A. Serum from 86 apparently healthy individuals was assayed using the Human IL-12/IL-23 p40 ELISA Kit (Catalog # RE1076H). **B.** Serum from 41 apparently healthy individuals was assayed using the Human High Sensitivity IL-17A(Interleukin 17) ELISA Kit (Catalog # RE2867HG).

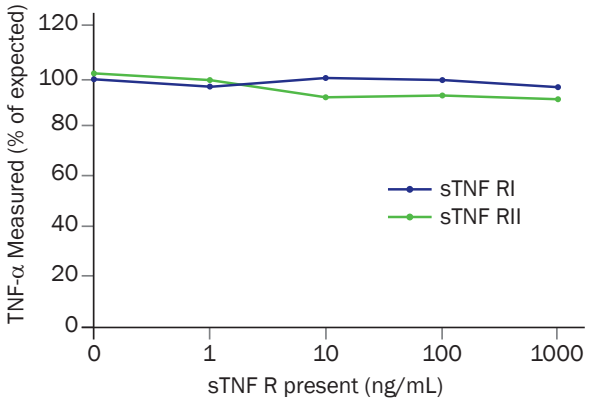
Linearity Experiments Identify False Positive Signals

False Positive ELISA Signals Can Be Identified by Assaying the Linearity of Dilution. Serial dilutions of a cell culture supernate were assayed for natural linearity using two different MMP-9 ELISA Kits.

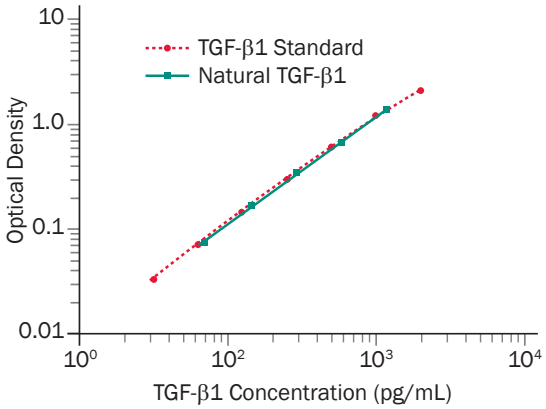
Diluted samples measured using the Human MMP-9(Matrix Metalloproteinase 9) ELISA Kit (Catalog # RE2796H) gave recovery results between 105–108% of the neat sample, supporting the linearity claim of the kit. In contrast, the target analyte was not detectable beyond the first dilution in samples measured with the second kit, indicating that the assay was producing a false positive signal. ND=Not detectable.

Sample Dilution	MMP-9 Kit	Competitor Kit
	Analyte Concentration Detected (ng/mL)*	
	5.26	19.63
1:2	104%	69%
1:4	106%	ND
1:8	103%	ND
Linearity claim	85–115%	69–128%

* Samples were diluted prior to the assay as directed in the product data sheet. All samples and dilutions were within the standard curve range.



Interference Testing of the Human TNF-α ELISA. TNF-α, at concentrations of 125–1000 pg/mL, was measured in the presence or absence of soluble TNF receptors (sTNF RI or sTNF RII) using the Human TNF-α ELISA Kit (Catalog # RE1060H). The results demonstrate that the presence of the soluble TNF receptors at concentrations up to 1000 ng/mL does not affect the TNF-α concentration determined using the ELISA Kit.



Reed Biotech Traditional conventions ELISA Kits Are Developed to Detect Natural and Recombinant Proteins. A serum sample containing activated human TGF-β1 was serially diluted (blue line) and compared to the TGF-β1 standard curve (red line). Results show that the Human TGF-β1 ELISA Kit (Catalog # RE10013) measures recombinant and natural TGF-β1 with equal effectiveness.

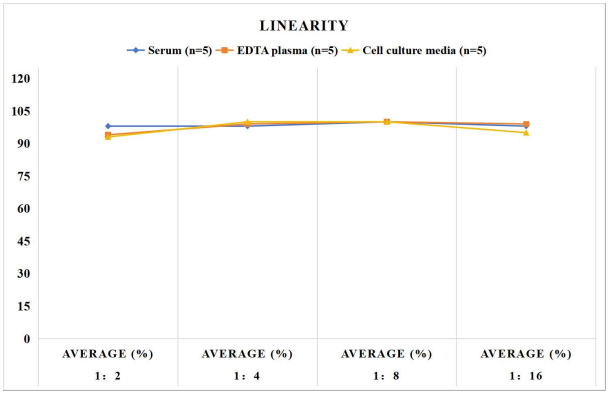
Learn more about identifying and eliminating false positive results. Review our application note.

What is the importance of ELISA controls?

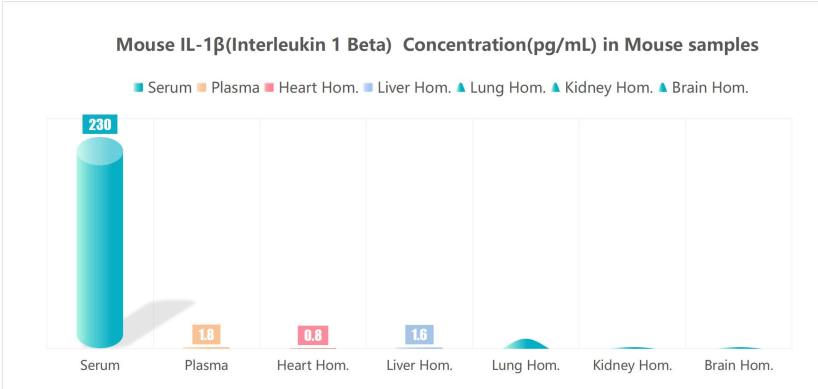
The importance of including ELISA controls, both positive and negative, in your immunoassay helps to verify that the assay was run properly and everything is performing accurately.

Positive ELISA Controls

A positive ELISA control can be a recombinant or natural sample that you know will be detectable in the assay. Positive controls help to show that a negative sample is truly negative. The standard curve is one form of positive control and you can compare your results to the standard curve data that is provided in your product insert. Reed Biotech also sells ELISA controls for the Traditional conventions ELISAs . Most Human Elisa kits have a lyophilized tri-level control with expected ranges that are validated by our Quality Control and our Mouse/ Rat ELISAs include one control in the kit. These are great when running multiple plates or when you have multiple users running the assay, to verify that values are all within the expected ranges.



Quantitation of Human IL-6 in High, Medium, and Low Controls. High (blue line), medium (Orange Line), and low (yellow line) controls are assayed with every manufactured lot of the Human IL-6 ELISA Kit (Catalog # RE3186H). Controls for the Human IL-6 ELISA Kit fall within acceptable ranges (gray bars) and remain consistent from lot to lot.



ELISA Spike Controls

When using complex sample matrices, it is also important to make sure that there is nothing present in the matrix that interferes in the assay. It is recommended to spike in recombinant or natural protein into your matrix and verify that the amount you spike in is what you read out.

Negative ELISA Controls

Negative controls help to verify that you are not obtaining any false positive results or non-specific binding. Use a sample that you know will does express the protein you are measuring. If you are quantitating a cell culture supernate, a good negative control would be to test your cell culture media.

Sample Preparations

Sample Collection & Storage

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated. (Some proteins require the presence of fetal calf serum for stability)

Plasma

Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Cell Culture Supernates

Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Cell Lysates

Solubilize cell in lysis buffer and allow to sit on ice for 30 minutes. Centrifuge tubes at 14,000 x g for 5 minutes to remove insoluble material. Aliquot the supernatant into a new tube and discard the remaining whole cell extract. Quantify total protein concentration using a total protein assay. Assay immediately or aliquot and store at ≤ -20°C.

Platelet-poor Plasma

Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. An additional centrifugation step of the plasma at 10,000 x g for 10 minutes at 2-8°C is recommended for complete platelet removal. Assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Serum

Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Saliva

Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, assay immediately or aliquot and store samples at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Urine

Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter. Assay immediately or aliquot and store at ≤ -20°C. Avoid repeated freeze-thaw cycles.

Human Milk

Centrifuge for 15 minutes at 1000 x g at 2-8°C. Collect the aqueous fraction and repeat this process a total of 3 times. Assay immediately.

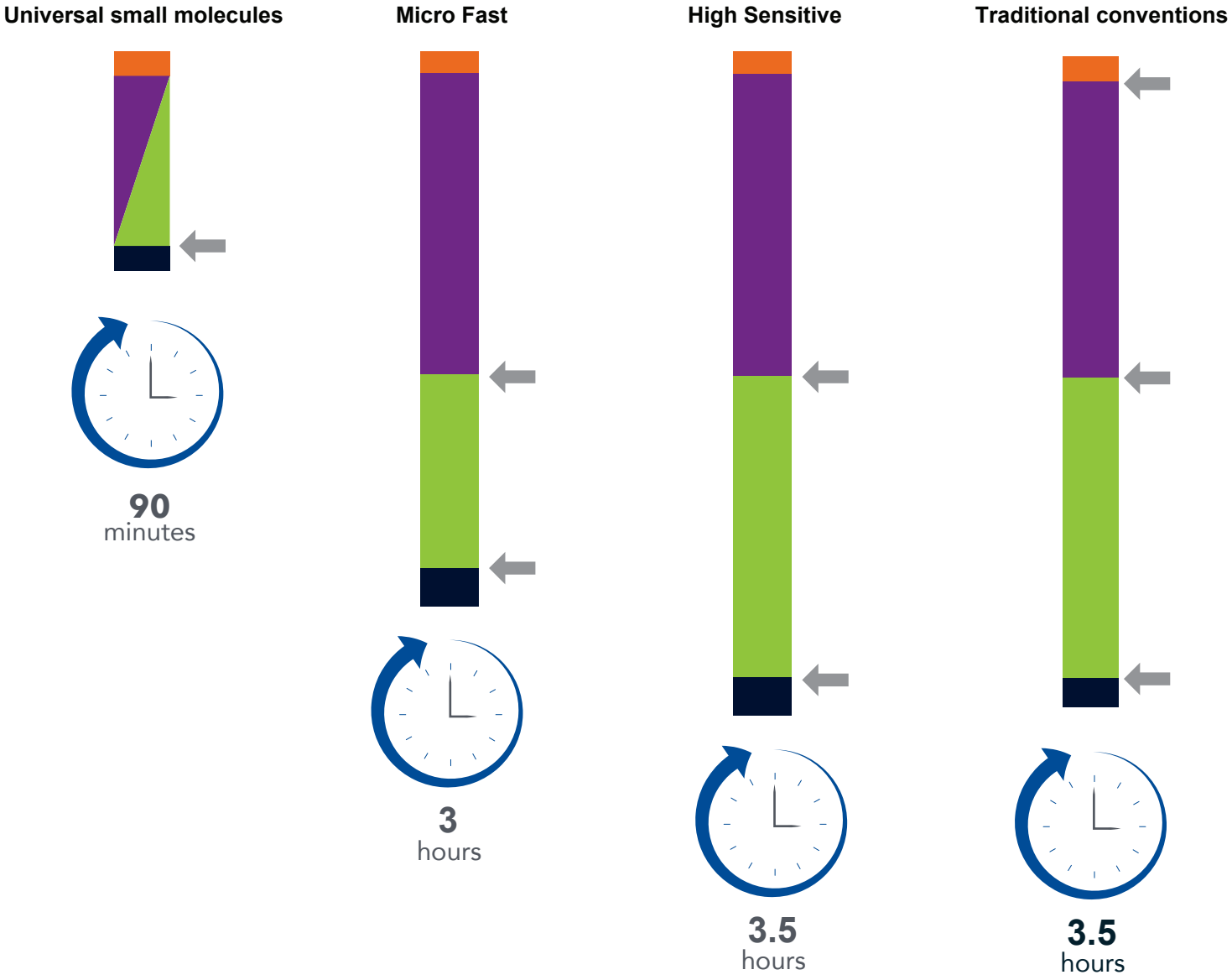
Tissue Homogenates

The preparation of tissue homogenates will vary depending upon tissue type. Rinse tissue with 1X PBS to remove excess blood, homogenized in 20 mL of 1X PBS and stored overnight at ≤ -20°C. After two freeze-thaw cycles were performed to break the cell membranes, the homogenates were centrifuged for 5 minutes at 5000 x g. The supernate was removed immediately and assayed. Alternatively, aliquot and store samples at ≤ -20°C. Avoid repeated

Tissue Lysates

Rinse tissue with PBS, cut into 1-2 mm pieces, and homogenize with a tissue homogenizer in PBS. Add an equal volume of RIPA buffer containing protease inhibitors and lyse tissues at room temperature for 30 minutes with gentle agitation. Centrifuge to remove debris. Quantify total protein concentration using a total protein assay. Assay immediately or aliquot and store at ≤ -20°C.

ELISA Assay Timelines



KEY

- Sample/reagent Prep
- Analyte Capture
- Detection Antibody Incubation
- Substrate Incubation
- Wash Step

Data Analysis: Calculation of Results

The values of the unknown samples are assigned in relation to the standard curve. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Always run ELISA samples in duplicate or triplicate This will provide enough data for statistical validation ofthe results.

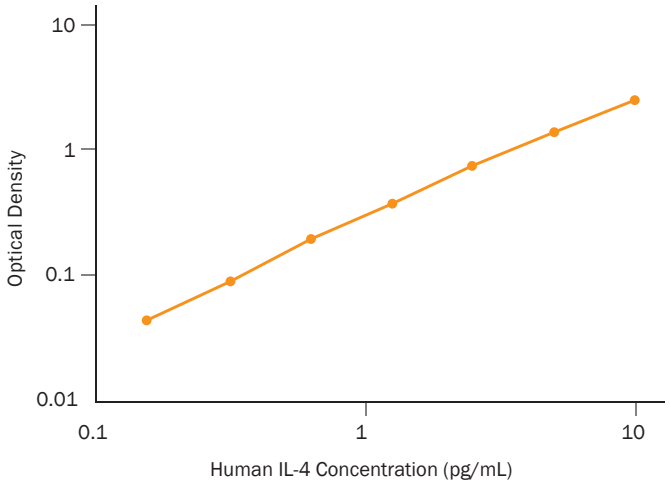
Average the duplicate or triplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.). The coefficient of variation (CV) of duplicates should be ≤ 20%.

Create a standard curve by reducing the data using computer software capable of plotting the mean absorbance (y axis) against the protein concentration (x axis). When possible, utilize the recommended data reduction method specified in the assay protocol.

If the recommended data reduction method is unavailable, it is recommended that various methods (e.g. linear, semi-log, log/log, 4 or 5 parameter logistic) be tried to see which curve best fits the data. One way to determine if the curve fit is correct is to backfit the standard curve O.D. values. To do this, first plot the standard curve.Next, treat standards as unknowns and interpolate the O.D. values from your standard curve. They should read close to the expected values (+/- 10%). Use the data reduction method that gives the best correlation value and backfit.

If software is unavailable, the data may be linearized by plotting the log of the concentrations versus the log of the O.D. on a linear scale. The best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

A representative standard curve is shown in the figure below from Human High Sensitivity IL-4(Interleukin 4) ELISA Kit (Cat# RE1055HG).



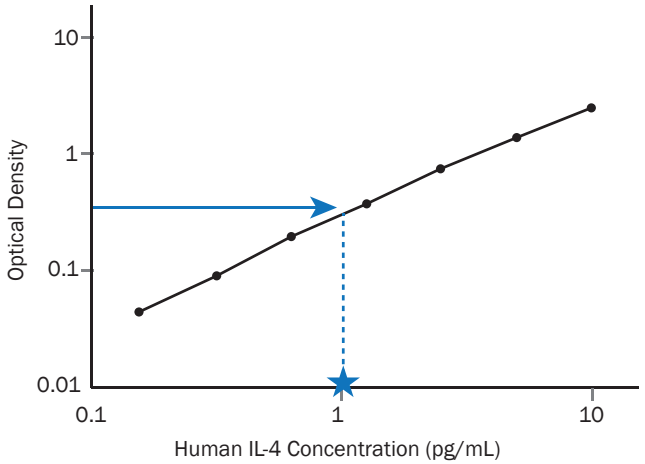
(pg/mL)	O.D.	Average	Corrected
0	0.051	0.059	-
	0.067		
0:156	0.101	0.103	0.044
	0.105		
0.313	0.148	0.149	0.090
	0.149		
0.625	0.246	0.251	0.192
	0.255		
1.25	0.431	0.432	0.373
	0.433		
2.5	0.798	0.804	0.745
	0.809		
5	1.407	1.418	1.359
	1.429		
10	2.485	2.498	2.439
	2.510		

Calculating concentration of target protein in the sample

To determine the concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

CV = standard deviation mean

Calculating CV is important as it can indicate any inconsistencies or inaccuracies in your ELISA results. The CV of duplicates should be ≤ 20%. A larger CV indicates greater inconsistency and possible error.

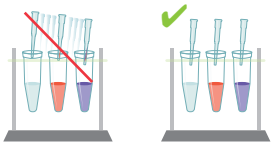
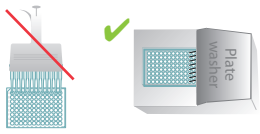
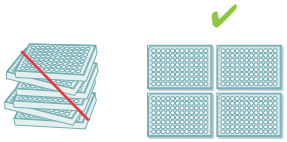


Calculating the coefficient of variation

The coefficient of variation (CV) is the ratio of the standard deviation to the mean, which is usually expressed as a percentage.

Best Practices and Techniques

While Reed Biotech builds ELISA kits to be robust in the hands of even inexperienced users, there are several tips and tricks that can help even the experienced user get the most from their assay.

	Make sure all reagents are brought to room temperature before using (unless instructed to keep them cold).
	If you are not going to run the entire plate, ensure that the remaining strips are sealed in the plate bag with the desiccant to prevent moisture from degrading the plate.
	For standards that are not single use, it is best to aliquot the remaining standard into smaller volumes and freeze. This allows you to avoid repeated freeze-thaws.
	Multichannel pipettes speed the ability to plate your standard and samples and lead to more consistent results.
	When pipetting, dispense liquid with the pipette tips held at an angle and not touching the bottom of the well.
	While it is not necessary to change your pipette tips between each replicate, it is recommended that you change them between different samples or standards to prevent contamination.
	It is highly recommended that a plate washer is used as manual plate washing can lead to higher backgrounds.
	When washing plates, either manually or with a plate washer, be sure to give the wash buffer time to work by adding a 30 second soak time in between washes.
	Pay close attention to the incubation times. As a general guide the incubation time should not vary by more than +/- 5 minutes per hour of incubation time.
	If the assay calls for incubation in a cold environment, at 2–8 °C, and you are running multiple assays, do not stack the plates on top of each other instead placing them individually on the shelf.

Reed Biotech® ELISA FAQs

What is included in a Elisa Kit?

Reed Biotech builds ELISA kits are a complete kit consisting of a precoated microplate, Conjugated Detection Antibody, Standard, Diluents, Substrate, Stop Solution, Wash Buffer, and plate sealers. They are fully validated ELISAs for the sample types listed in the specific datasheet. They have been exhaustively tested for superior quality.

How many samples can be assayed in a ELISA kit?

Reed Biotech builds ELISA kits will run the standard curve and 40 samples in duplicate. Please refer to the datasheet for details on each kit.

What samples can be tested in the kit?

Typically the Reed Biotech kits are validated for sera, two types of plasma, and cell culture supernate. However, the samples validated in an ELISA can vary from product to product. The product datasheet and product-specific web page states all sample types that have been validated for use with the ELISA kit. These are the only samples for which we can support the claims. References may exist for other sample types. See the "Sample type" tab on the product-specific webpage for any published references citing the use of the kit with an alternate sample type. Unclaimed sample types should be validated by the customer.

Has this kit ever been tested with my sample type?

Fortunately, Reed Biotech has routinely tested many sample types such as tissue homogenates or bronchoalveolar lavage for ELISA kits. This does not mean that the ELISA kit is not suitable for other sample types. One will need to perform a spike and recovery study to determine if an unvalidated sample type will work with a particular kit. To perform a spike and recovery experiment, one should divide a sample into two aliquots. In one of the aliquots, the user should spike in a known amount of the kit standard. A dilution series is performed comparing the spiked versus the unspiked sample. Generally, samples with expected recovery and linearity between 80–120% are considered acceptable. This method may be used to validate any sample type that has not been evaluated by Reed Biotech. For a more detailed spike and recovery protocol, please contact Technical Service. Note: Acceptable ranges should be determined individually by each laboratory.

Why can I not detect any of my samples?

You will be able to quantify samples down to the lowest point on the standard curve. In some cases, the standard curve does go down low enough to detect normal samples. You can check the Sample Values section in your kit booklet to find out what kind of sample values we obtained from apparently healthy individuals. You may also want to review the literature to find out if there is an established normal range for your target. It is important to recognize that assay platforms and manufacturers differ in their calibrations for their unique assay products and reported measurements may not directly correlate.

Can I extend the standard curve (in either direction)?

Reed Biotech cannot support kit results outside the stated range under any circumstances. A specific range was chosen because of confidence in the reproducibility of the assay.

Why doesn't the assay range extend to the stated sensitivity?

Sensitivity is the lowest measurable value that is statistically not equal to zero. It is calculated based on the signal of the background and the inherent variability of the assay. It is commonly determined by taking the mean O.D. plus two standard deviations from 20 zero replicates. This value is converted into analyte concentration from the standard curve. The low standard is the lowest possible point at which Reed Biotech feels confident that the value is in the linear portion of the standard curve and, therefore, quantifiable. Values which are greater than the sensitivity can be distinguished as separate from the background or the noise of the assay, however the confidence level for reporting these values is lower than if the sample values fall within the standard curve range.

Why is a sample dilution necessary in some kits?

There are primarily two reasons for dilutions. In some assays most samples read above the standard curve, thus requiring a dilution for analyte levels to fall within the range of the assay. A second reason for dilution is to limit interference due to factors in complex matrices.

Won't addition of Assay Diluent cause further dilution of the sample?

Since the assay diluent is added to all wells, standards and specimens are treated equally. Therefore, sample concentration can be read from the standard curve without adjusting for this dilution.

Is there enough Calibrator Diluent for all of my sample preparations?

The kits are designed with enough calibrator diluent to ensure that the vast majority of samples fall within the indicated range of the assay. Should you find that there is not enough diluent provided in the kit to dilute your samples, you have at least two options. Option 1) Samples can be diluted in two steps. The initial dilution in culture medium and a final dilution, of at least 1:10, into the Calibrator Diluent provided in the kit. Option 2) For a nominal charge, you can purchase additional diluent provided the same lot included in the kit is still available. Contact Technical Service for more information.

My diluents appear to contain precipitate. Is this ok?

Due to saturating amounts of some buffer components, some of the Assay Diluents contain a light to heavy precipitate. In these instances, it will be noted in the specific protocol booklet. If it is not noted in the protocol booklet, please contact Technical Service.

The assay protocol specifies to use the shaker at 500 rpm. This is too fast for my shaker. Is this correct?

This is 500 rpm with a 0.12 orbit. If the plate shaker has a larger orbit, then 500 rpm will be too fast. Reed Biotech recommends the ThermoFisher Model # 4625 microtiter plater shaker. Assays requiring shaker incubations have been optimized for performance with these shaker specifications only.

Are controls available for kits?

Reed Biotech offers tri-level control sets for the Human Traditional Colorimetric ELISA Kits, High Sensitive ELISA kits, ELISA Auxiliary Reagent and Micro Fast ELISA Kits Please inquire for specific ordering information.

What is the stability of supplemental ELISA controls?

Controls are assigned an expiration date of 6 months from date of receipt. They are to be used once and discarded. If the lyophilized controls are stored properly, it is possible that they will remain stable for an extended period of time, although we have not conducted extended stability testing. The controls have not been tested for stability after reconstitution.

I used your recombinant protein as a control in the corresponding ELISA kit. Why am I seeing discrepancy in mass values?

First, a large dilution is required to place the recombinant protein on the standard curve range. Typically this is a dilution from µg/mL to pg/mL. Any dilution step can introduce inaccuracy and the larger the dilution step the greater the potential for error. Any pipetting error or mis-calibrated pipet can result in apparent over- or under-recovery. Second, Reed Biotech immunoassays have been developed to measure a level of protein captured by one antibody and detected by a second antibody. This measurement is calibrated to standards established when the kit was initially developed. The protein determination of these initial standards became the Master Calibrators to which all new standards are formulated. This provides Reed Biotech immunoassay kits with consistency between manufacturing lots. In general, we would expect +/- 25% recovery of the amount stated on the vial when using the Traditional Colorimetric ELISA to determine a protein concentration. There may be slight differences in the immunologically recognizable mass between lots of protein, so the apparent concentration provided on the vial may vary from lot-to-lot when measured in the ELISA. If you are using proteins to make controls, it is better to value assign the mass based on measurement in ELISA and not use the mass on the vial when setting control levels.

Why must I use polypropylene tubes for standard curve dilutions in certain assays?

Certain proteins or analytes will bind to glass and polystyrene, but do not readily bind to the polypropylene tubes.

Why are my wells green after adding the stop solution?

This happens when the substrate in the well does not completely mix with the stop solution. After addition of the stop solution, tap the plate gently or place on a shaker until the mixture in the wells turns yellow.

Why is there brown precipitate in my wells after addition of the stop solution?

This is due to incomplete washing after the HRP-labeled detection antibody (or streptavidin-HRP) incubation. When HRP is present during the substrate and subsequent stop solution additions, an orange-brown or brown precipitate is observed. This may be remedied by the addition of a 30 second soak on each wash step followed by complete removal of all liquid in the wells.

What is a competitive ELISA?

In the competitive immunoassay approach, also termed labeled analyte technique, there exists a competition between the endogenous unlabeled antigen and an exogenous labeled antigen for a limited amount of antibody binding sites. Therefore, a decreasing signal indicates higher concentrations of the analyte being measured.

What is a sandwich ELISA?

A sandwich ELISA uses an immobilized capture antibody specific for the analyte of interest in a sample. After the analyte is bound to the immobilized antibody, a labeled secondary antibody specific for the analyte is used for detection. The analyte is “sandwiched” between the two antibodies. The sandwich ELISA is extremely sensitive, and the values obtained are quantitative when compared with a standard curve.

Can a partial Traditional Colorimetric ELISA plate be used?

The Reed Biotech ELISA plates have removable strips of wells. Unused wells may be removed from the plate, returned to the foil pouch containing the desiccant pack, and stored at 2–8°C for up to one month.

Can I stop an assay at any point, extend an incubation time or change the suggested incubation temperature?

Reed Biotech has optimized the assays for both incubation times and temperatures. Each kit has only been validated for the protocol described in the kit datasheet. We cannot guarantee the performance of our kits when the protocol has been altered in any way.

Can reagents from different kits be interchanged?

Assay Diluent(s), Calibrator Diluent(s), and substrate may be interchanged if they have the same part number AND lot number. Reed Biotech does “whole kit QC” which means that we cannot support the use of reagents from other lots or sources being substituted into an assay. Plates and Conjugate cannot be interchanged under any circumstance.

Why do I need to use a 4-PL curve fit for generating my standard curve?

Reed Biotech develops and QCs most of our Traditional Colorimetric ELISA Kits using a 4-parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the concentrations versus the log of the O.D., and the best fit line can be determined by linear regression. This procedure will produce an adequate but less precise fit of the data.

Why am I seeing high variability between sample duplicates?

The two main reasons for high variability in an assay is related to pipetting & washing technique.

Troubleshooting your Reed Biotech® ELISA

Problem	Possible Cause	Solution
No signal or low signal	Reagents added in incorrect order, or incorrectly prepared	• Repeat assay
		• Check calculations, standard reconstitution, etc.
	Standard has been damaged (if there is a signal in the sample wells)	• Check that standard was handled according to directions. Avoid vortexing.
		• Use new vial
	Incorrect incubation conditions	• Check incubation conditions were for the specified length, at the appropriate temperature, and shaker specifications were met if required.
	Incorrect filters	• Check specified signal and correction wavelengths in the protocol
	Incorrect Storage/Handling	• Check that kit was stored properly according to conditions indicated on the box label
Too much signal – whole plate turned uniformly blue	Insufficient washing/washing step skipped – unbound peroxidase remaining	• See washing procedure
	Substrate Solution mixed too early and turned blue	• Substrate Solution should be mixed and used immediately
	Plate sealers or reagent reservoirs reused, resulting in presence of residual HRP. This will turn the TMB blue non-specifically	• Use fresh plate sealer and reagent reservoir for each step
	Work surface cleaned with bleach	• Residual bleach fumes can oxidize TMB and cause non-specific high signal
Standard curve achieved but poor discrimination between points (low or flat curve)	Plate not developed long enough	• Increase Substrate Solution incubation time
		• Use recommended time
	Incorrect procedure	• Eliminate modifications, if any
	Improper calculation of standard curve dilutions	• Check calculations, make new standard curve
	Insufficient washing	• See washing procedure
		• If using an automatic plate washer, check that all ports are clean and free of obstructions, add a 30 second soak step and rotate plate halfway through the wash
	Plate sealer reused	• Use a fresh plate sealer for each step
Poor Duplicates	No plate sealers used	• Use plate sealers
	Insufficient washing	• See washing procedures
		• If using an automatic plate washer, check that all ports are clean and free of obstructions
	Variations in incubation temperature	• Avoid incubating plates in areas where environmental conditions vary
	Variations in protocol	• Adhere to the same validated assay protocol
	Variation in pipetting	• Ensure all pipette tips are securely fastened and dispensing consistent volumes
		• Establish use of either forward or reverse pipetting for entirety of the assay
	Improper shaker	• Check that shaker orbit and speed meet specifications indicated in the kit insert. Any splashing on the plate sealer or foaming of liquid in the sample can also result in poor precision.
	Saliva contamination	• Wear a mask to avoid contamination
Poor assay to assay reproducibility	Plate sealers reused	• Use fresh plate sealer for each step
	Improper calculation of standard curve dilutions	• Check calculations, make new standard curve
		• Use internal controls
No signal when a signal is expected, but standard curve looks fine	No cytokine in sample or levels below assay range	• Repeat experiment
		• Reconsider experimental parameters
		• Obtain fresh samples, minimize freeze-thaw cycles
		• Use enzyme inhibitors
	Sample matrix is masking detection	• Dilute samples at least 1:2 in appropriate diluent, or preferably do a series of dilutions to look at recovery
		• If specified in the kit protocol, the assay may only recognize the sample after specific treatment. Follow any sample treatments specified in assay insert.
Samples are reading too high, but standard curve looks find	Samples contain cytokine levels above assay range	• Dilute samples further and run again
Very low readings across the plate	Incorrect wavelengths	• Check filters/reader
	Insufficient development time	• Increase development time
Green color develops upon addition of stop solution when using streptavidin-HRP	Reagents not mixed well enough in wells	• Tap plate
Edge Effects	Uneven temperatures around work surfaces	• Avoid incubating plates in areas where environmental conditions vary
		• Use plate sealers
Drift	Interrupted assay set-up	• Assay set-up should be continuous – have all standards and samples prepared appropriately before commencement of the assay
	Reagents not at room temperature	• Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

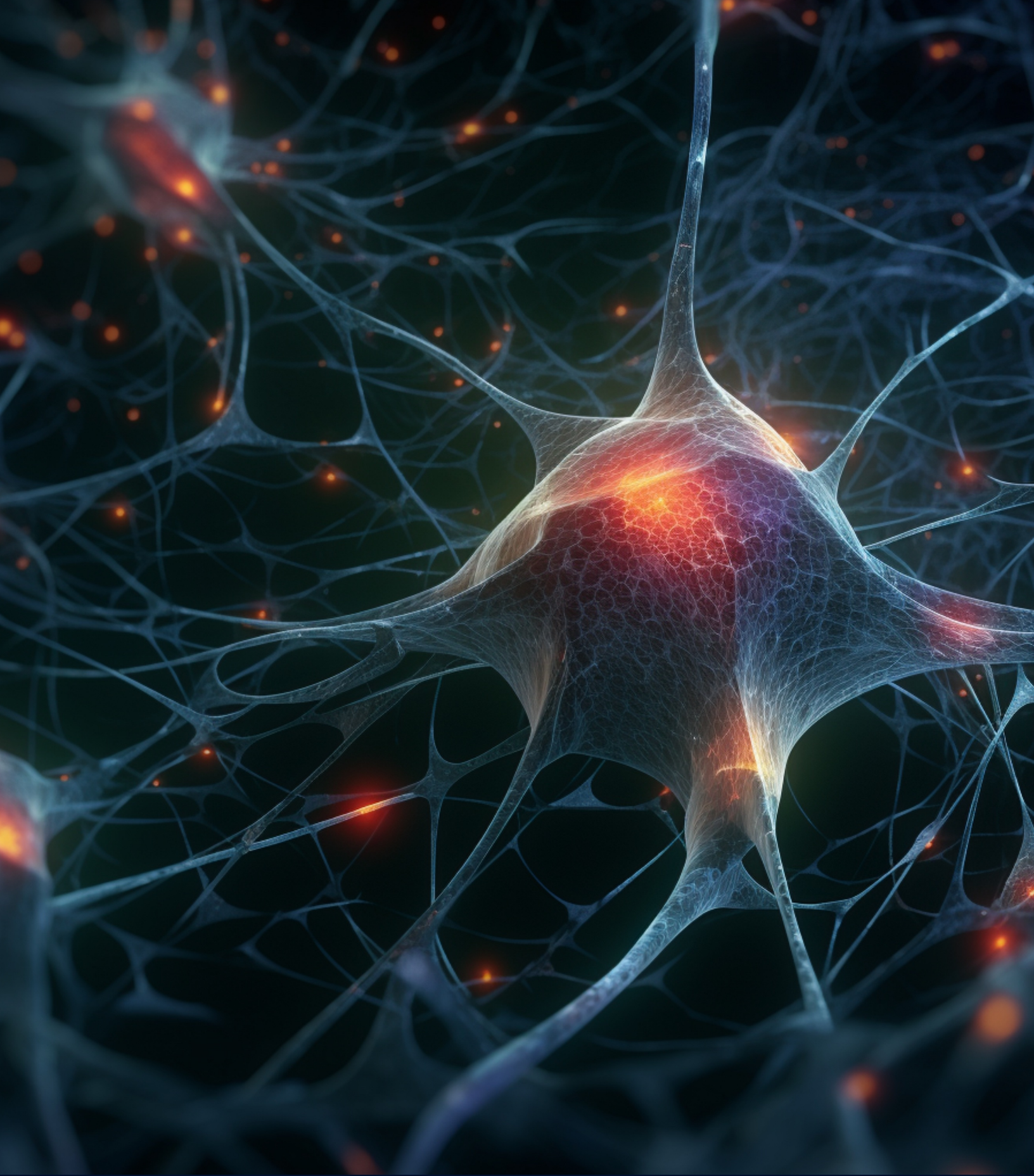
ELISA Kit Offerings

Traditional Colorimetric Sandwich ELISA Kits	
Species	**# of Kits**
Human	458
Mouse	140
Rat	53
Porcine	15
Canine	15
Multi-Species	7
Cynomologus Monkey	1
Rhesus Macaque	1
Viral	1

Other species Kits	
Species	**# of Kits**
Human	732
Mouse	297
Rat	61
Canine	17
Porcine	13
Feline	10
Equine	8
Bovine	5
Rabbit	5
Multi-Species	5
Cotton Rat	4
Guinea Pig	3
Primate	2
Viral	1
C. botulinum	1
Viral	1

Supplemental
Other scientific research tool Products

Antibody	
Name	**Applications**
Histone H3 Mouse Monoclonal Antibody(Mix-mA™)	WB, IHC, IF, IP
α skeletal muscle actin Mouse Monoclonal Antibody	WB, IP, IHC
Histone H3 Mouse Monoclonal Antibody(8F7)	WB
β II tubulin Mouse Monoclonal Antibody	WB
Transferrin Mouse Monoclonal Antibody(7F4)	WB
FH Fumarase Mouse Monoclonal Antibody(2B11)	WB, IF
Flotillin-1 Mouse Monoclonal Antibody(6C10)	WB
Progesterone Receptor Mouse Monoclonal Antibody	IHC, IF
Vimentin Mouse Monoclonal Antibody(1A7)	WB, IHC
COX2 Cyclooxygenase 2 Mouse Monoclonal Antibody	IHC
Lactoferrin Mouse Monoclonal Antibody(Q100)	ELISA
CD68 Mouse Monoclonal Antibody(12E2)	IHC
Histone H2B Mouse Monoclonal Antibody(Mix-mA)	WB, IHC
eNOS Mouse Monoclonal Antibody(Mix-mA)	WB, IHC
β III tubulin Mouse Monoclonal Antibody(Mix-mA)	WB, IHC
Caspase 8 Mouse Monoclonal Antibody(2G12)	WB, IHC



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