VeriQuant™ High-Sensitivity Immunoassay Guide

Next-Generation Approaches to Protein Quantification

Where Boundless Ideas
Converge with Cutting-Edge Science

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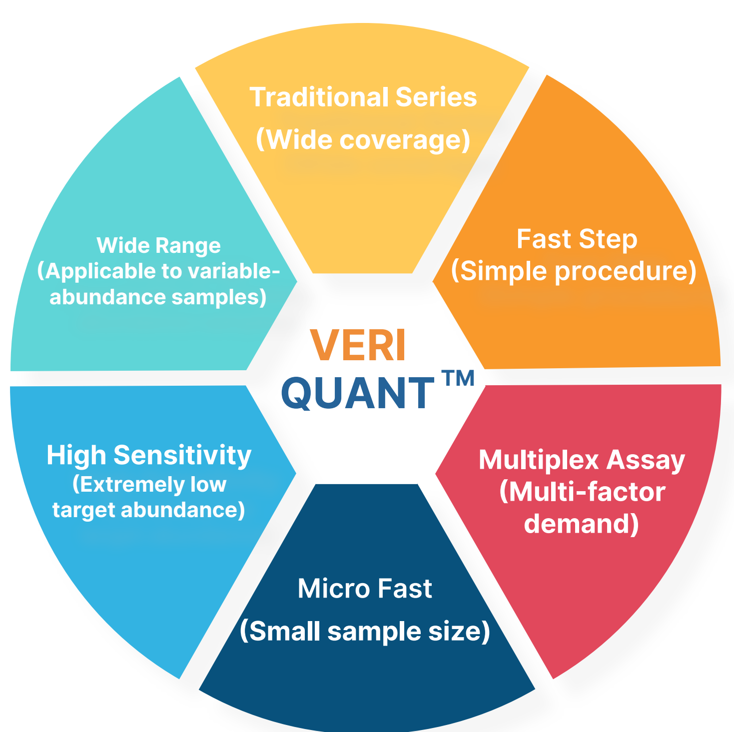
VeriQuant™ – Next-Generation Approaches to Protein Quantification

ReedBiotech "VeriQuant™" Immunoassay Kit | Brand-new ready-to-use test reagents, accurate target measured with micro samples, bid farewell to cumbersome operations, unlock a new solution for efficient and sensitive detection

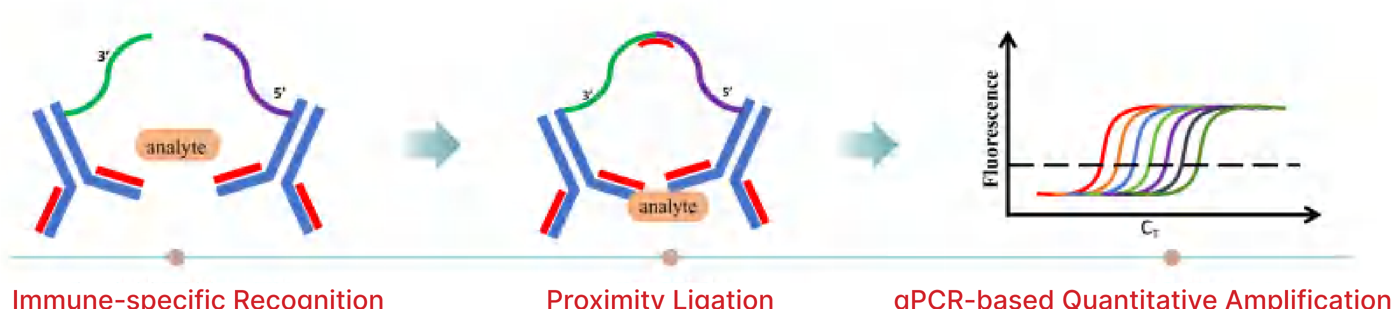
- **User-Friendly Operation:** Streamlined protocol with a wash-free format to minimize operational errors
- **Micro Sample Requirement:** Requires only 1-5 μL of sample, ideal for scarce or precious samples
- **High Efficiency & Time-saving:** Total assay time is 2 hours, with only 0.5 hours of manual operation, significantly boosting throughput
- **Universal Consumables:** Compatible with standard qPCR consumables. (Reed Biotech Select Sets are available for separate purchase)
- **Superior Performance:** With a femtogram-level sensitivity, the detection range spans 6-log dynamic range
- **Cost-Effective:** No expensive proprietary instruments required; compatible with standard qPCR systems

All in one



What is the VeriQuant™ Immunoassay?

VeriQuant™ Immunoassay Kit is based on Proximity Ligation Assay (PLA) technology. It utilizes a pair of target-specific antibodies, each conjugated to a 3' primer and a 5' primer, respectively. When the antibody pair binds to two distinct epitopes on the target protein, the primers are brought into close proximity. A template strand for amplification is generated only upon specific protein binding: the 3' and 5' primers hybridize with a complementary connector probe and are ligated by DNA ligase. The resulting DNA template is then exponentially amplified via TaqMan Quantitative PCR (qPCR). This mechanism enables ultra-high sensitivity detection while maintaining a wide detection range.

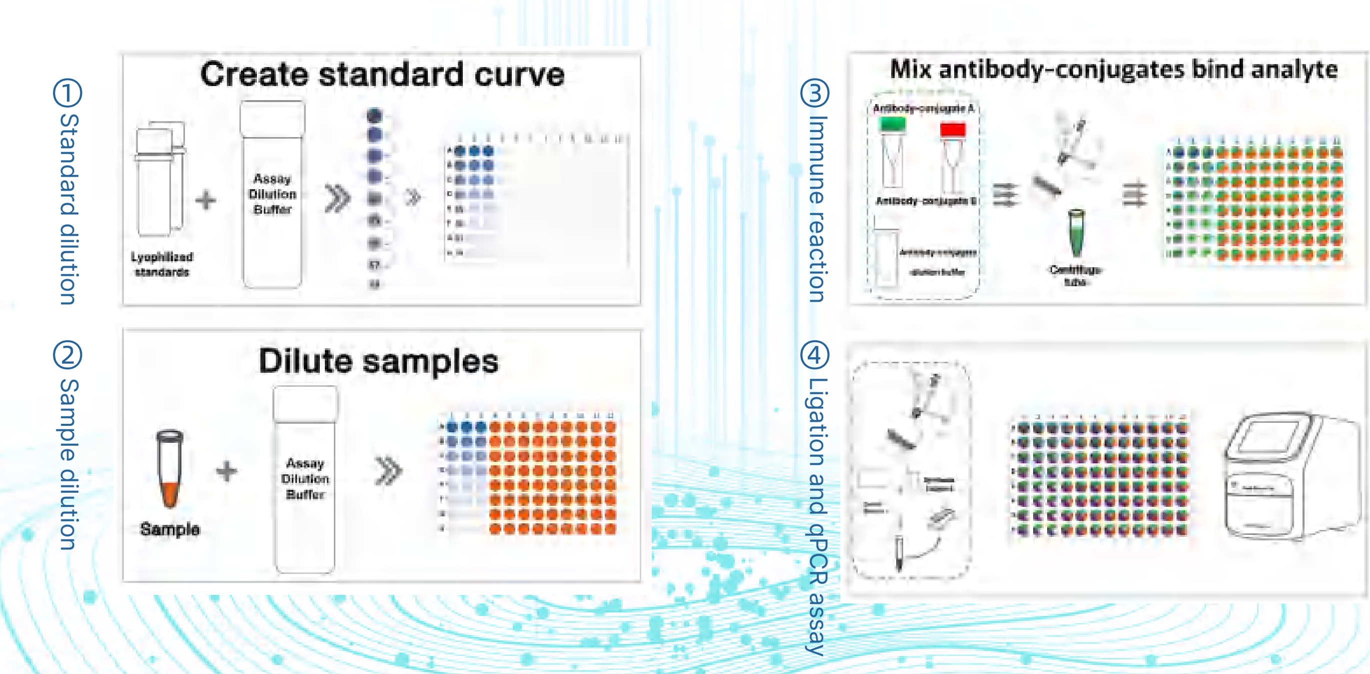


(a) Two monoclonal antibodies against distinct epitopes are conjugated with oligo probes, then form a sandwich complex with the antigen.

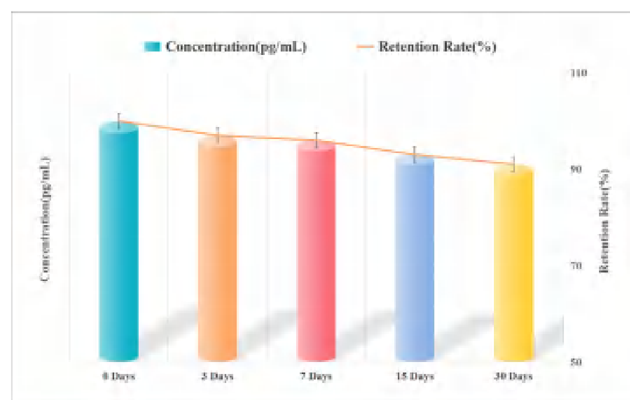
(b) Following antigen recognition by the paired antibodies, the conjugated oligonucleotides are spatially proximate, and generate an amplifiable dsDNA template under the enzymatic action.

(c) qPCR amplification is then performed. CT values correlate inversely with protein concentration, and sample target concentrations are calculated via standard curve.

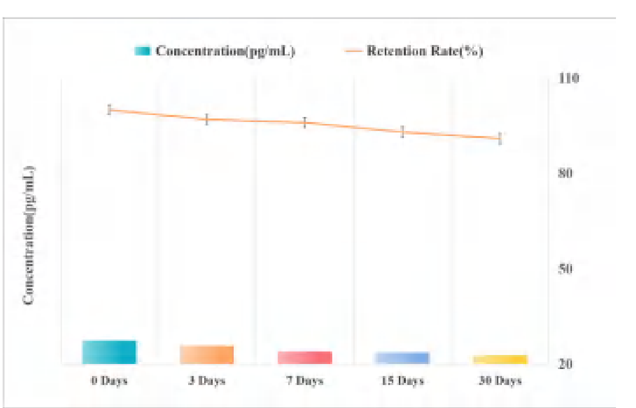
Assay Workflow



Stability Test



High Concentration (n=26)

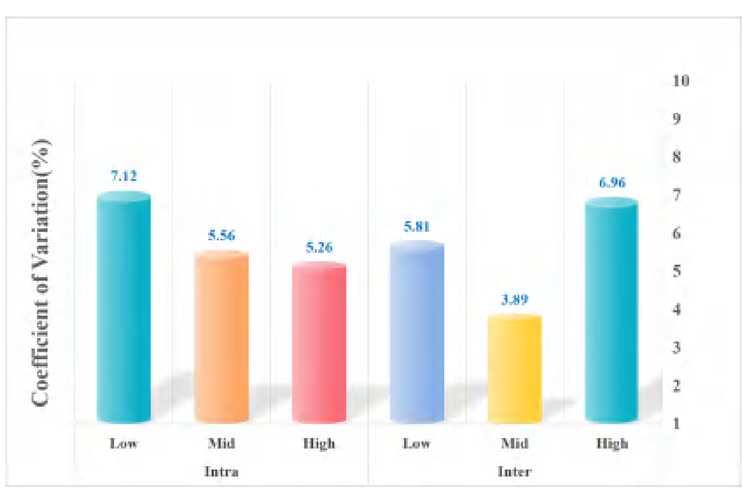


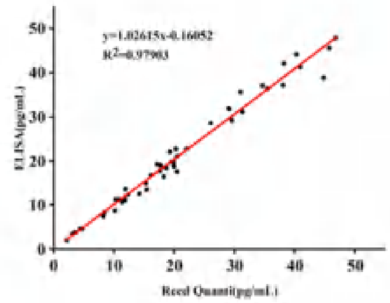
Low Concentration (n=17)

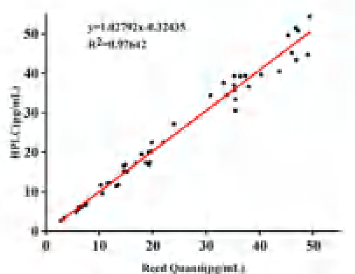
An accelerated stability test was conducted by storing the kit at 37°C for 3, 7, 15, and 30 days. Human IL-6 serum samples at high concentration (n=26) and low concentration (n=17) were tested, the results demonstrated that the activity recovery rates were all ≥90%.

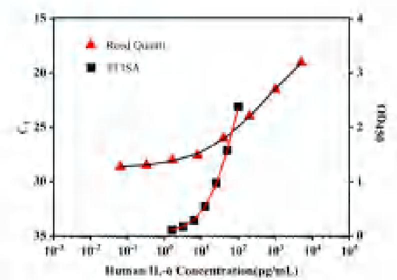
Intra-batch & Inter-batch Variation

Mouse IFN-γ kits were randomly selected to assess precision. Intra-batch precision (n=8) and inter-batch precision (n=5) were determined by testing samples with known high, medium, and low concentrations, batch difference from 4.77% to 7.13%.









*Simultaneous testing of Human IFN-γ in human serum samples (n=47) with traditional ELISA kit: correlation coefficient r=0.9893

*Simultaneous testing of Human IL-10 in human serum samples (n=56) with LC-MS: correlation coefficient r= 0.9765

VeriQuant ™ Human IL-6
Detection range: 0.064–5000pg/mL
Sensitivity: 0.0209pg/mL

Full-stack self-developed ReedLytix™ conjugation system addresses the inability of traditional conjugation to achieve site-specific and fixed-ratio modification

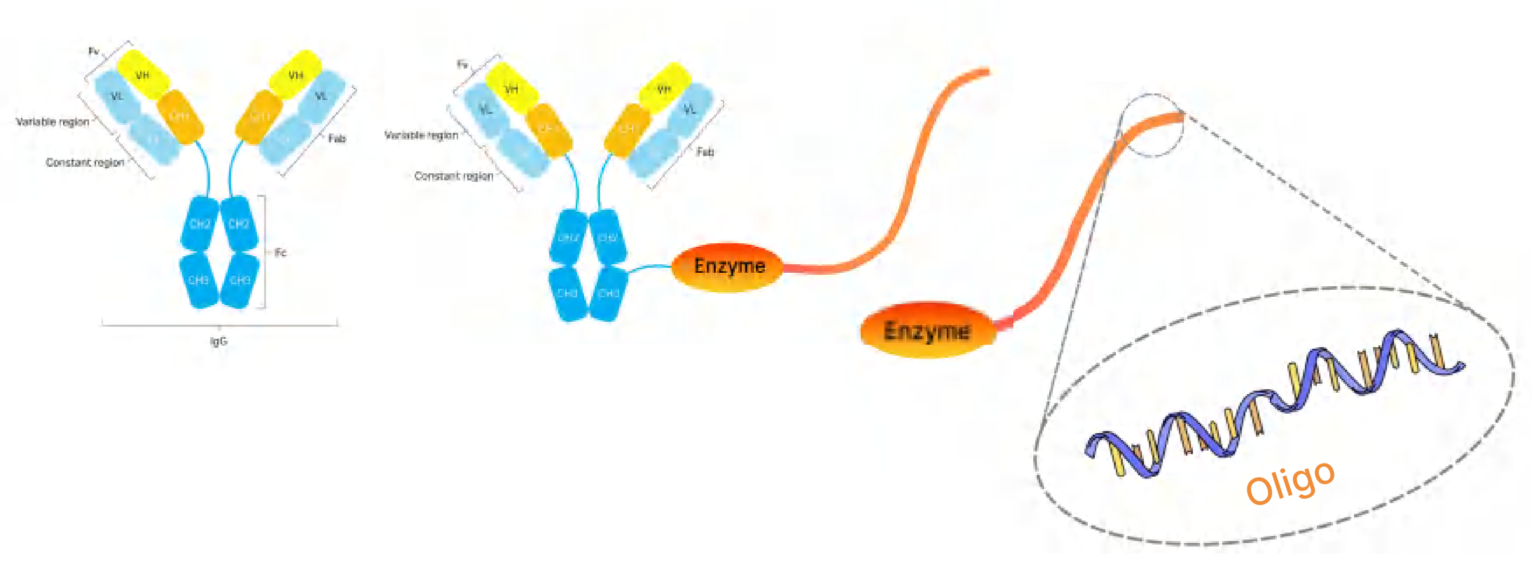
ReedBiotech full-stack self-developed polymerase Quanti Mixture and upgraded Synthesis Enzymes buffer system can easily achieve fg/mL-level protein quantification; Greatly simplify the process, enhance efficiency and consistency. Integrate the "connection" and "extension" reactions in the same reaction tube, and complete all chemical reactions with just one incubation without the additional pipetting.

Triple Specificity

Specific Antibody ----- Rigorously screened from our proprietary screening platform with years of technical accumulation

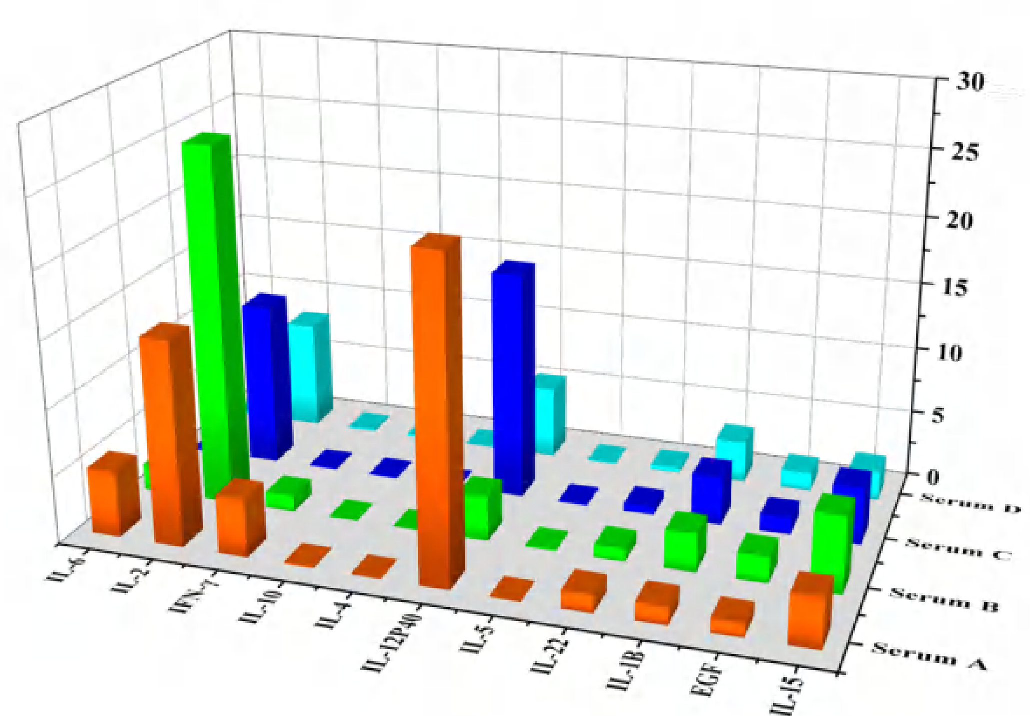
Specific Conjugation ----- Self-developed ReedLytix™ enables site-specific and ratio-specific labeling

Specific Labels ----- Self-designed high-specificity Oligo for easy exponential signal amplification

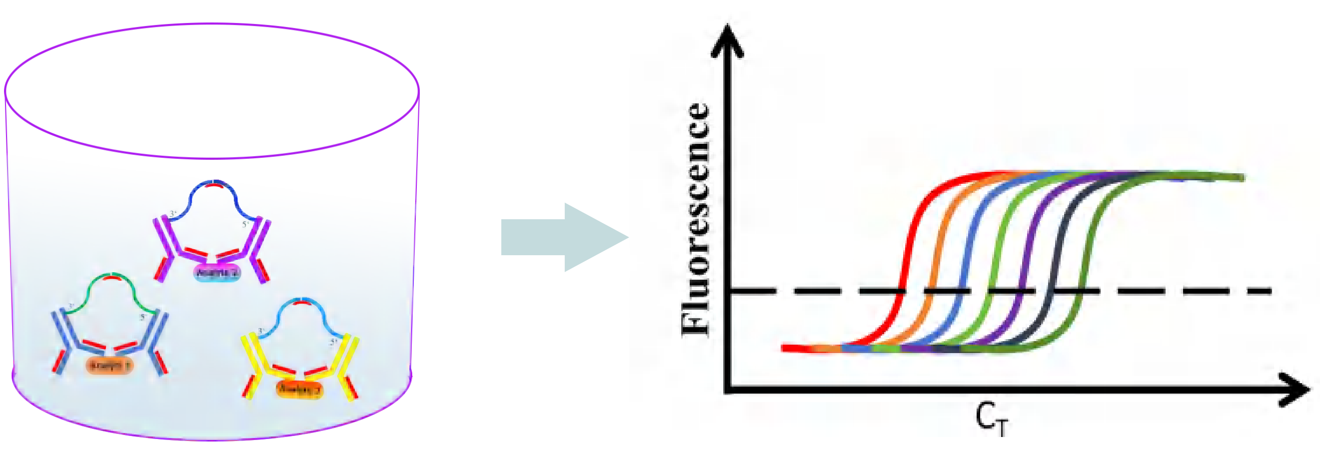


Multi-Detection based on the VeriQuant™ Platform

VeriQuant™ Multiplex Assays deliver fast, high-efficiency multi-protein analysis in a single well. Compared to ELISA, this multiplex approach generates significantly more data from each sample—be it plasma, serum, or tissue culture supernatant. Most importantly, it allows for more comprehensive protein signaling studies using your precious samples.

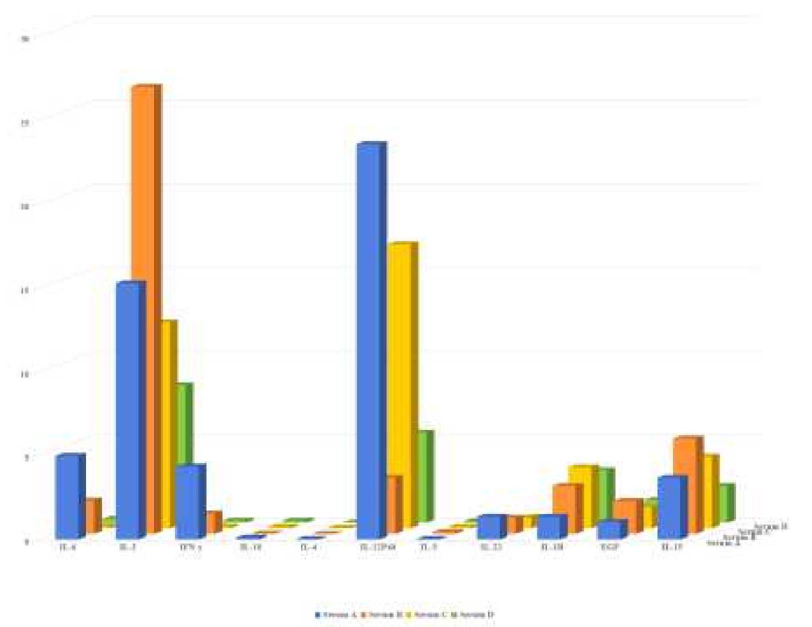


How VeriQuant™ Achieves Synchronous Detection and Precise Identification of Multiple Target Proteins in a Single Reaction System: The kit contains multiple sets of specific target-matched antibody pairs, each conjugated to a unique oligonucleotide (Oligo) probe. Upon binding to distinct epitopes on the target protein, the paired antibodies bring the adjacent nucleic acid probes into close proximity, enabling them to form a DNA template via ligase action. Signal amplification is then achieved through qPCR. By utilizing fluorescence-specific probes, VeriQuant™ identifies multiple target signals, and the concentration of each target protein is precisely quantified based on the resulting Ct values.



Rigorous assay validation helps ensure consistent, reliable results

Four serum samples were analyzed using the VeriQuant™ Cytokine Assay in a single experiment with a mouse inflammatory factor 11-Plex multiplex assay. Eleven different cytokines and growth factors were measured in the serum samples from four different individuals. Data analysis was performed using the OriginLab system



Th1/Th2 Cytokine Panel (3-plex)		
Cat. No. VQM003		
GM-CSF	IFN gamma	IL-12 p70

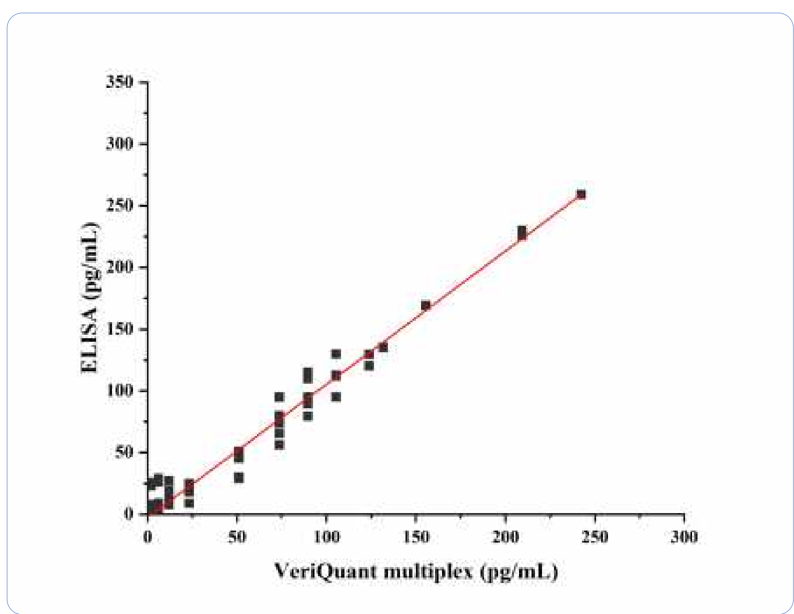
Cytokine Panel 1C (6-plex)		
Cat. No. VQM006		
TNF alpha	TNF beta (LTA)	IL-31
IL-1RA	IL-15	IL-7

Growth Factor Panel 1A (6-plex)		
Cat. No. VQM09A		
BDNF	SCF	NGF beta
EGF	PDGF-BB	FGF-2

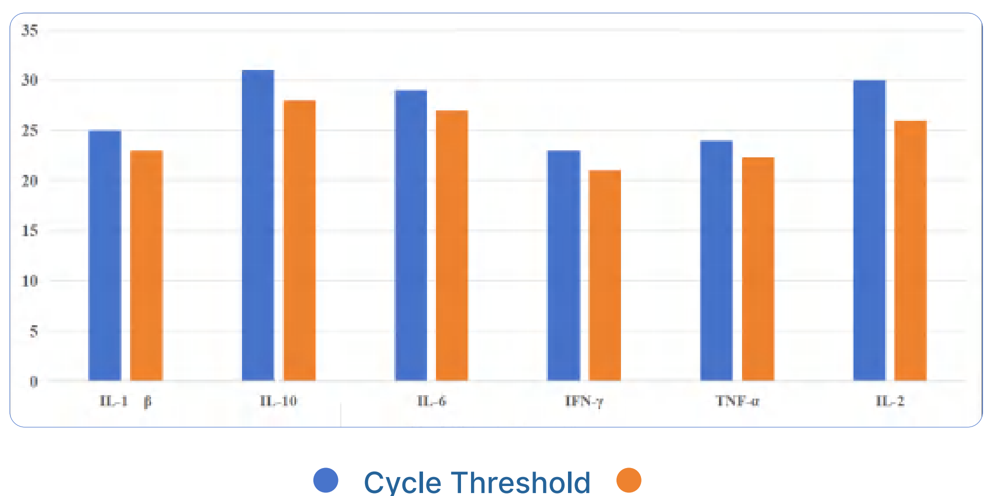
ReedBiotech multiplex kits undergo rigorous quality testing and are correlated to our ELISA kits if available to offer confidence that switching between protein analysis platforms will provide comparable analytical results.

Specifications for kit development

Specifications	Description
Benchmarking to ELISA	Correlates with ELISA data
Recovery	Tested on serum and plasma
Sensitivity	Physiologically relevant levels, <10 pg/mL (based on detectable signal >2 SD above background)
Precision	Inter-assay CV: (between assays) Intra-assay CV: (within assays)
Specificity	Cross-reactivity tests are performed with other analytes and antibodies
Linearity of dilution	High coefficient of correlation between sample dilutions and expected concentration over the range of the assay
Parallelism to natural samples	Recombinant standards are compared to natural samples to ensure equivalency



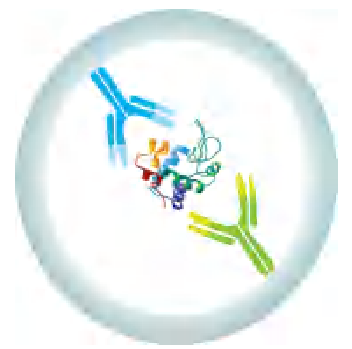
Strong correlation between ELISA and VeriQuant™ multiplex assay results for the detection of mouse IL-6 in tissue culture supernatant



Cycle Threshold

Precision verification: The blue and orange bars represent the mean values of 24 replicates measured at different times. The CV for all tested samples was <10%. The VeriQuant™ Human Cytokine Multiplex Assay Kit 6-Plex Panel (Cat. No.: VQM06D) was used.

What is an ELISA ?



Enzyme-linked immunosorbent assay (ELISA) is a common immunoassay method used to quantify the level of a specific target in a sample. Samples routinely used in ELISA include serum, plasma, cell culture supernatant, cell lysate, saliva, tissue lysate, and urine.

ELISA assays are typically run in 96-well microplates that are pre-coated with a capture antibody specific for the analyte of interest. After incubation with the 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. Subsequently, a substrate solution is added, generating a signal that is proportional to the amount of bound analyte. ELISA can be performed in various different formats. Descriptions and diagrams of these can be found in the next section.

The Highest-Quality ELISAs Available

ReedBiotech®, 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 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
- Packaging Available Long-term storage at 2-8°C

What's the Core of your Immunoassay?

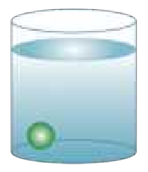
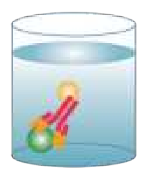
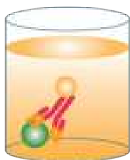
Your results matter, so what's inside your immunoassay should too. ReedBiotech® antibodies and proteins are the core of every ReedBio immunoassay platform. Our antibodies and proteins are highly specific, manufactured in-house to ensure reproducibility and tested for suitability on every application 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

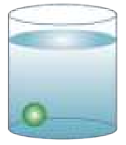
- Fast and simple protocol

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

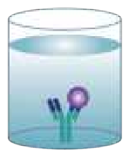
- Amplification using a secondary antibody

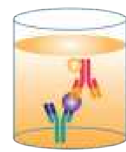
Disadvantages

- 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

- The biotin-avidin system amplifies the signal, greatly improving sensitivity and reducing background

Disadvantages

- The operation steps are a bit more complicated.

Competitive ELISA

Competitive enzyme-linked immunosorbent assay (ELISA) is commonly used for small molecules such as hormones and peptides. A target antigen conjugate is coated onto the microplate, and the detection antibody along with the sample is added simultaneously. The target antigen in the sample and the immobilized solid-phase antigen compete for binding to the detection antibody. The more antigen present in the sample, the less antigen binds to the capture antibody. After the substrate is added, the signal generated is inversely proportional to the amount of protein 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

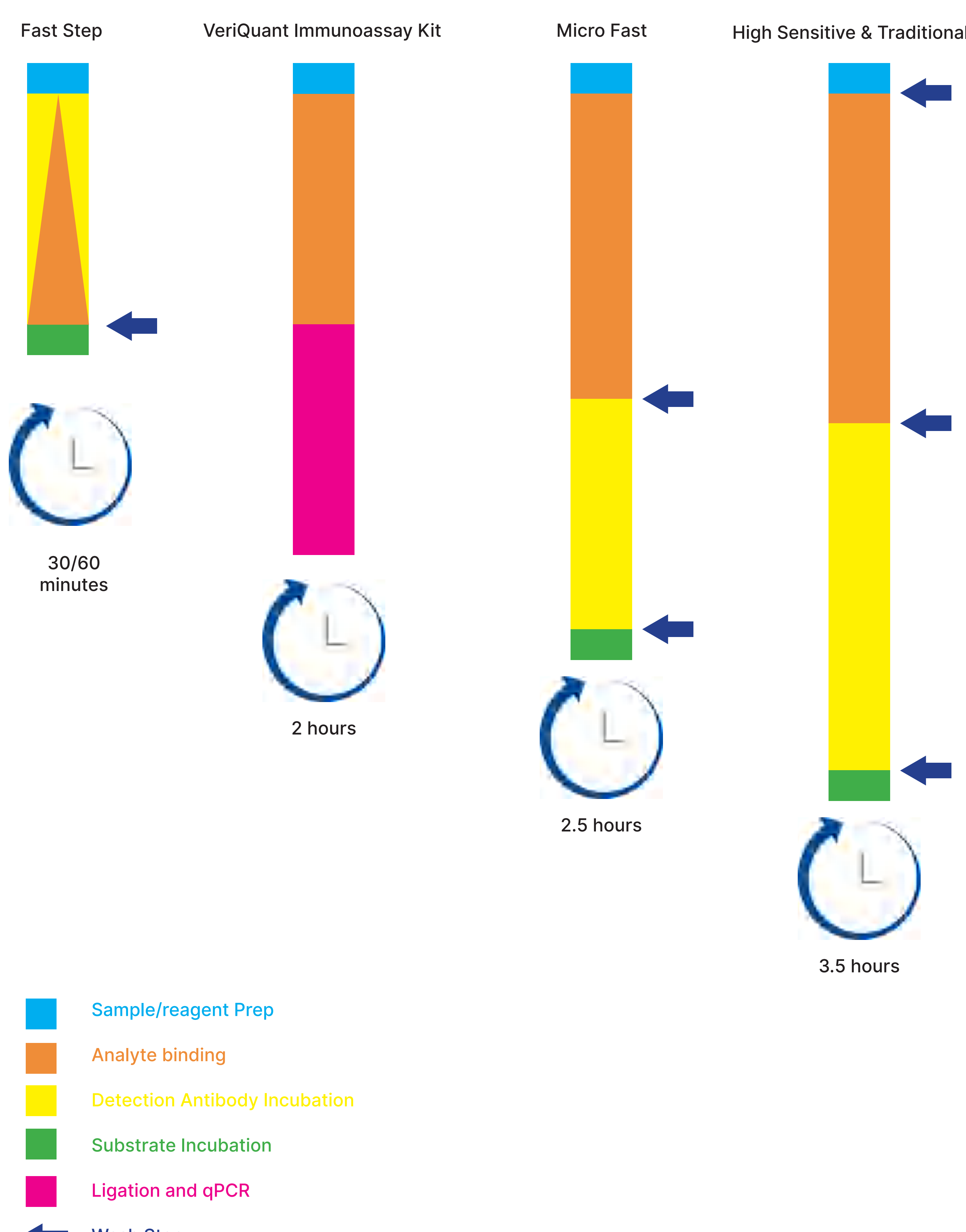
Why use an ELISA over other techniques?

There are many different immunoassay platformsavailable 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?

Parameter	VeriQuant	Fast Step ELISA	High Sensitive ELISA	Micro Fast ELISA	Traditional ELISA
Format	96T/384T	96T(88 samples)	96T(88 samples)	96T(88 samples)	96T(88 samples)
Benefit	*Wide range *No washing step *Simple and convenient operation *Flexible reaction system *fg-level sensitivity *1-5μL sample volume *Compatible with qPCR instruments and consumables	*Super time-saving	* Highest Sensitivity * Extremely low sample consumption (5μL stock solution + 95μL diluent)	* Broad species availability * Time-saving * Low sample requirement	* Multiple target options * Low CVs, high sample adaptability * Time-saving compared to competing products
Sample Volume	1~5μL	50μL	100μL	25μL	100μL
Assay Time	2 Hours	Minimum 0.5 Hours	3.5 Hours	2.5 Hours	1.5/3.5 Hours
Number of Analytes	1~6	1	1	1	1
Pre-coated	Ready-to-use	Ready-to-use	Ready-to-use	Ready-to-use	Ready-to-use

Comparison of Immunoassay Timelines



New Generation ELISA Manufacturer: **ReedBiotech®** ELISA

Key Ccomponents

- Pre-coated 96-well Microplate(Dismountable)
- Concentrated Biotinylated Antibody
- Calibrated Immunoassay Standard
- Concentrated HRP conjugate
- Concentrated wash buffer (25X)
- Biotinylated antibody diluent
- Standard & sample diluent
- Certificate of Analysis
- Conjugate diluent
- Substrate solution
- Stop solution
- Product manual
- Plate sealers

Traditional ELISA Kit

Traditional ELISA kits are complete,ready-to-use kits and are the gold standard for single-analyte detection. These kits can be used to measure a wide variety of molecules, including cytokines, chemokines, growth factors, proteases, and more.

Micro Fast ELISA

Micro Fast ELISA (MF® Micro Fast Sandwich ELISA Kits) allow for the rapid quantification of target proteins within 150 minutes. By optimizing the assay protocol to reduce sample volume and experimental steps, these ready-to-use kits enable you to accomplish more experiments using less sample and less time

High Sensitivity ELISA

High Sensitivity ELISA (HS® High Sensitivity Sandwich ELISA Kit) is an upgraded version of the traditional ELISA. By combining signal amplification with high-quality antibody pairs, it increases detection sensitivity by 5–50 times, achieving pg/mL or even fg/mL levels. Designed specifically for low-abundance biomarkers such as cytokines, hormones, and tumor markers, it is ideal for early disease screening and low-abundance protein research.

Fast Step™ ELISA

Fast Step™, the one-step ELISA, is a simpler and more precise rapid ELISA product developed by ReedBiotech based on conventional ELISA. It optimizes the traditional three-step sandwich assay into a minimalist one-step process, reducing sample loading volume by half, shortening the operation time to 1.5 hours, and cutting the washing steps from three times to once. This greatly improves sample utilization efficiency and overall experimental efficiency for researchers

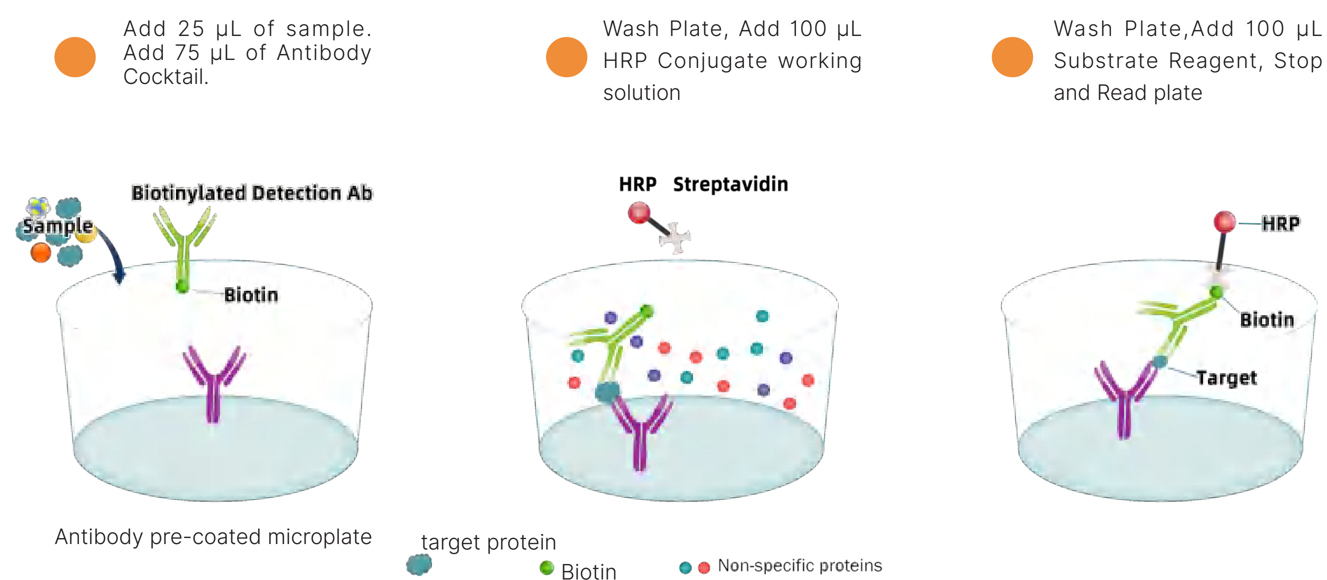
Uncoated ELISA

ReedBiotech's self-developed uncoated ELISA kit comes with a complete set of basic reagent components for ELISA experiments. It is suitable for research and testing customers with mature experimental experience who can independently establish a complete ELISA experimental system, making it an optimal solution for efficiently reducing experimental consumable costs. This product features a flexible and practical reagent combination, offering high freedom of use and strong adaptability. Compared to ready-to-use pre-coated ELISA kits, it offers higher cost-effectiveness and serves as an economical and practical high-quality alternative

Micro Fast® Colorimetric Sandwich ELISA Kit

Compared to the traditional three-step method, the Micro Fast ELISA Kit optimizes the sample loading volume from the traditional 100μL to 25μL, fundamentally solving the problem of scarce precious samples; it also condenses the three steps into two steps, reducing the number of plate washes and greatly improving the experimental efficiency of the experimenter.

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. ReedBiotech® ELISA kits excel in this area and the Micro Fast ELISAs are no different.

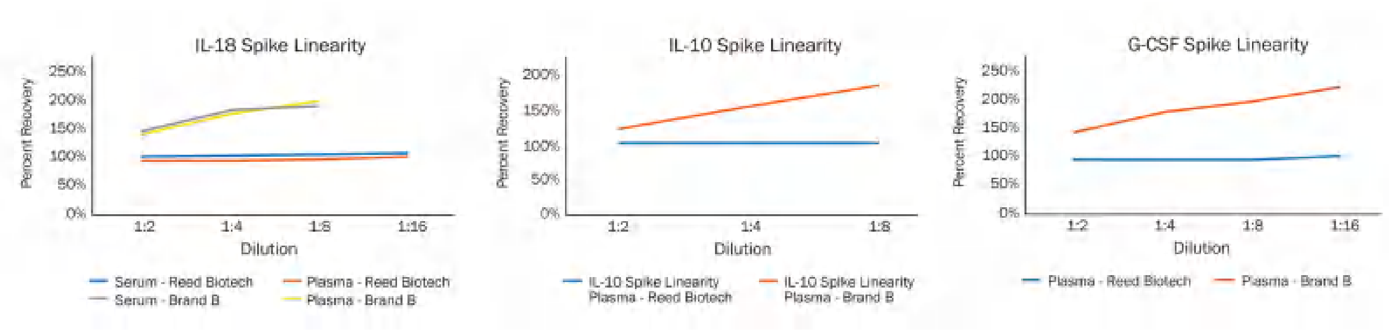


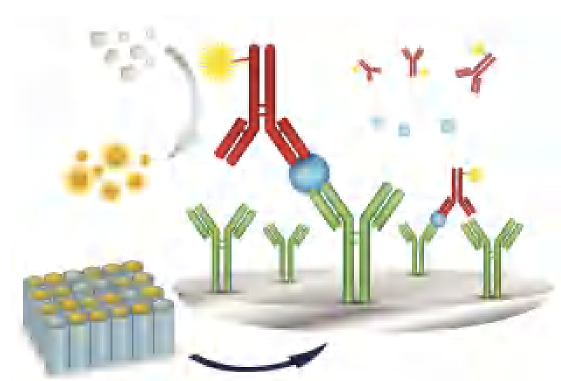
Figure 1. The IL-18 Micro Fast ELISA has superior linearity to Brand B. At dilute concentrations, Brand B is susceptible to matrix effects.

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

Figure 3. The ReedBiotech® G-CSF Micro FastTM has superior linearity to the Brand B counterpart.

Uncoated ELISA and antibody pair kits

ReedBiotech's independently developed uncoated ELISA kits come complete with all basic reagent components required for ELISA experiments. They are suitable for research and testing customers with mature laboratory experience who can independently establish a complete ELISA system, serving as an optimal solution for effectively reducing experimental consumable costs. Featuring a flexible and practical reagent formulation with high operational flexibility and strong adaptability, these kits offer a higher cost-performance ratio than finished pre-coated ELISA kits, making them an economical and ideal choice.



Advantages of uncoated ELISA kits

Preprocessing and optimization - The matching antibody pairs and the reagents are optimized for ELISA development.

Multi-specifications selection - Purchase according to your needs by selecting package sizes, including 5, 10 and 20 plates per kit.

Complete auxiliary reagent system - including all essential reagents and buffers that limit variability (common reagents such as washing buffer solution and termination solution).

Economy - Cost-effective and help maximize your research results.

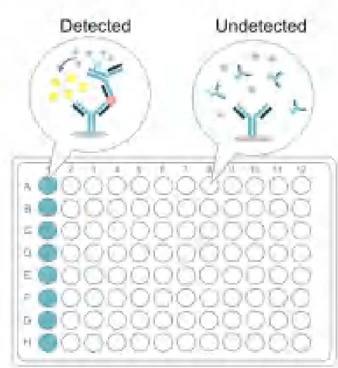
Flexible targets - matched antibody pairs can be used for ELISA or in conjunction with other immunoassay platforms.

Customized packaging - Integrated buffer kits can be sold separately if required for ELISA development.

No need to avoid light - Streptavidin HRP is easy to operate to offer the ultimate experience.

Unified reagent - Coated antibody diluent, blocking liquid and detection antibody diluent, HRP diluent, four in one, to avoid multiple change of reagents.

Kit Components



① Pretreatment to capture antibody
② Pretreatment detection antibody
③ Recombinant protein standard
④ Streptavidin-HRP
⑤ 96-well plate
⑥ Sealing film
⑦ Substrate solution
⑧ Stop solution
⑨ Detection buffer (sample diluent)
⑩ ELISA plate coated buffer solution
Concentrated washing buffer solution
&Product manual

Increase the speed of your research

Flexible packaging of 5 or 20 plates, to meet the needs of multiple sample experiments, more cost-effective.

Carefully selected and validated antibody pairs greatly reduce the waste of time and money in experimental optimization.

Corrected experimental standards to reduce experimental differentiation.

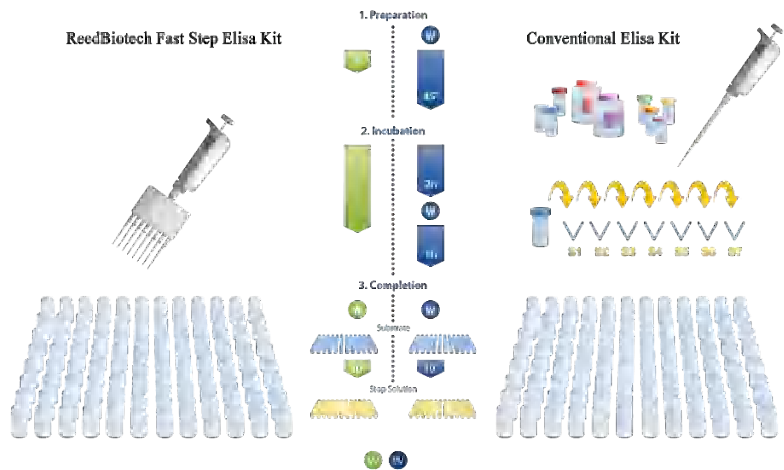
Suitable for a variety of research platforms.

Kit provides biotinized antibody detection and streptavidin-HRP, suitable for both chemiluminescence and colorimetric detection methods.

A wide range of uncoated kits, covering a wide range of biological processes and different molecules of sample types.

Fast Step™ One-Step Elisa kit

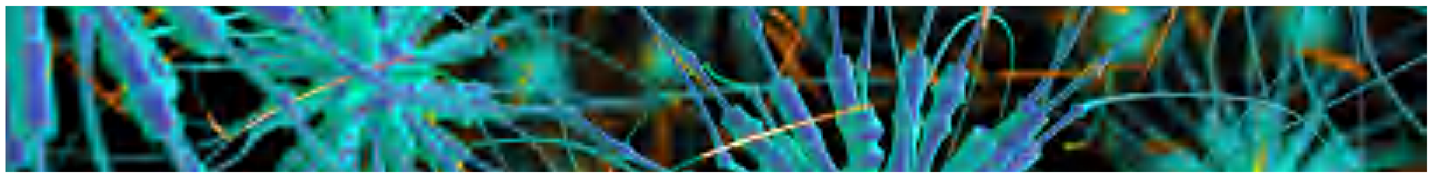
ReedBiotech's one-step ELISA kit features a streamlined workflow, reducing the inconvenience of multiple preparation and incubation steps. Traditional ELISA kits come with pre-coated plates that only offer capture antibodies, requiring sequential addition of samples and detection reagents. In contrast, the Fast Step ELISA kit utilizes biotin antibody and HRP complex coupling technology. Compared to conventional kits, it reduces the experimental process and operation time, Besides, it avoids the reagent consumption, which makes it more convenient for the majority of scientific research users



Only 30 minutes to complete the test

To reduce the number of steps helps to save time and minimize discrepancies. ReedBiotech uses high quality raw materials to utilize the latest Fast Step^{TM} ELISA kits with only 5 steps, However, performing a conventional ELISA requires 8 steps. As a result, with Fast Step^{TM} ELISA Kit technology, the overall manual processing time of an experiment can be reduced to about 30 minutes.

High Precision--only one addition of samples, antibodies & HRP, reducing manual operation means fewer errors and ensuring consistent results.
Good repeatability--sample addition is carried out in a closed space to avoid Cross-reactivity and make the detection result more accurate.
Save time--Minimum operation time with 30 minutes, adding standard products/samples can be tested, single cleaning procedure, improve the detection efficiency.
Reduce costs--reduce labor costs and time costs, no need to add antibodies and detection reagent steps multiple times, reduce the use of reagent consumables.

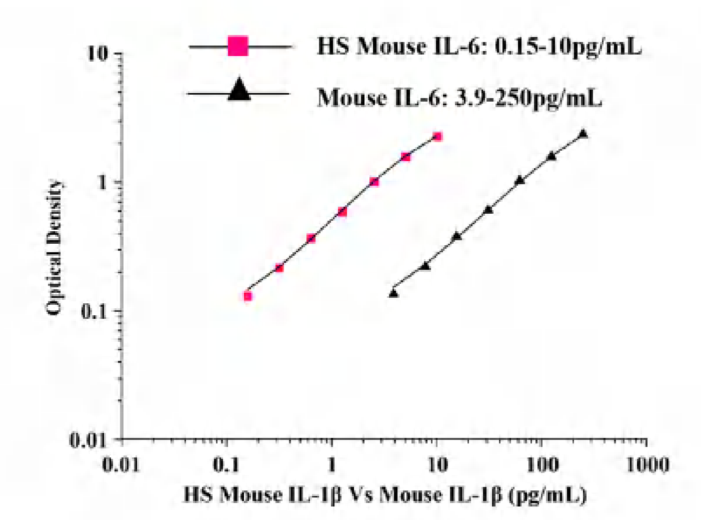


HS®High Sensitivity Sandwich ELISA Kit

The high-sensitivity immuno detection kit is a new series that offer researchers high-performance detection without the need for specialized equipment. Utilizing biotin-streptavidin signal amplification (BAS) technology, this kit employs antigen-antibody complexes for the detection of analytes, featuring exceptionally high signal detection and amplification capabilities, enabling highly sensitive protein quantification. Compared to traditional methods, this kit can detect lower levels of protein with minimal sample consumption, allowing for the maximization of precious or limited sample resources.

Product Feature

---Highest sensitivity: Measuring Proteins at the pg/mL or even fg/mL Range, which is 5–50 times higher than that of traditional ELISA, enabling accurate quantification of extremely low-abundance biomarkers.
---High specificity: Uses high affinity pair antibodies with extremely low crossreactivity, effectively reducing nonspecific interference and false positive risks.
---Excellent repeatability: Intra-assay and inter-assay coefficients of variation (CV) are < 10%, with stable signals, suitable for accurate quantification of low concentration samples.



High-sensitivity kits and conventional kits were used to quantitatively detect IL-1β in mouse samples. Control readings had to be within the set range of the mean ± 2 standard deviations.
Compared with conventional Mouse IL-1β, the sensitivity of high-sensitivity Mouse IL-1β was increased by 25 times.

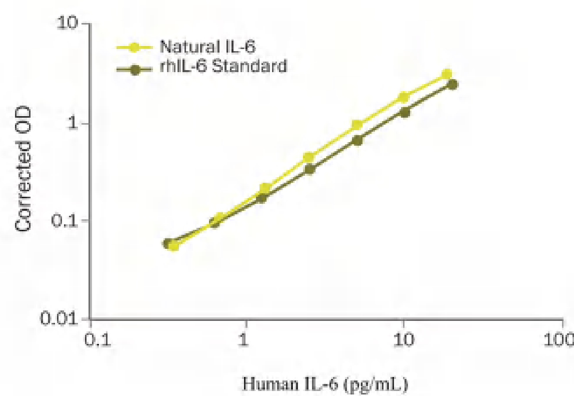
OD values of the standard curve for Mouse IL-1β.Using Mouse IL-1β High Sensitivity ELISA System (Item No. RE1074MG)

Ensuring ELISA Performance and Consistency

Accurate Detection of Natural Proteins

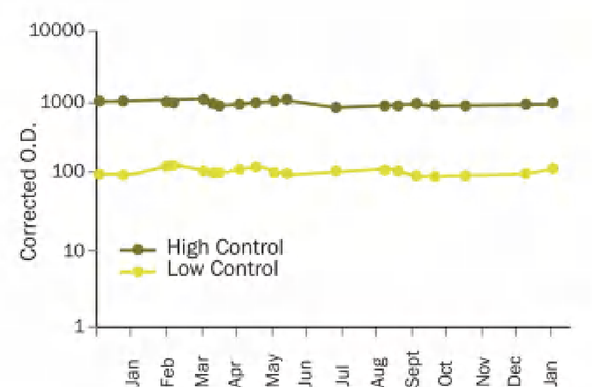
Antibody pairs recognize the supplied recombinant standard and the natural proteins in biological samples in a parallel manner, confirming that this kit can be used to measure the relative mass values of the natural analyte. ReedBiotech has determined the ideal standard curve range for each assay, ensuring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

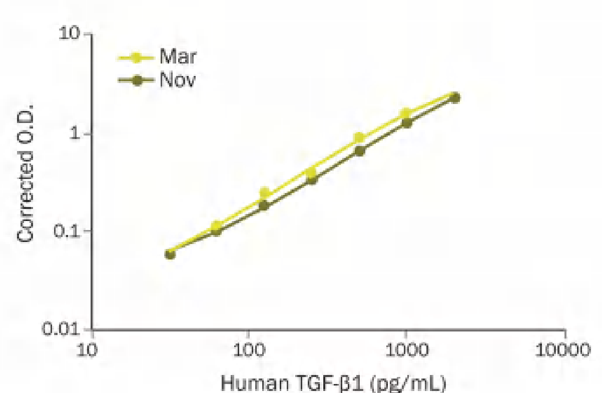


Confirmed Lot-to-Lot Consistency

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.

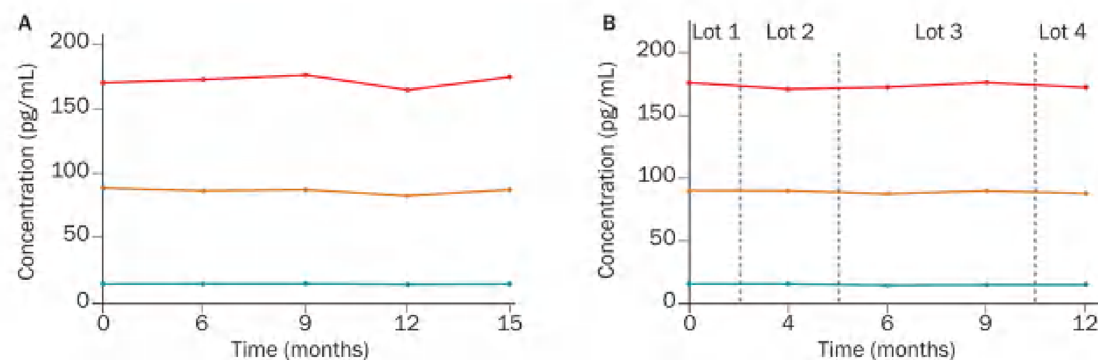
Precision & Reproducibility: Providing Confidence in Your Results

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.

2) determine if two results are the same or different. Precision is measured as a coefficient of variation (CV) from the mean value.

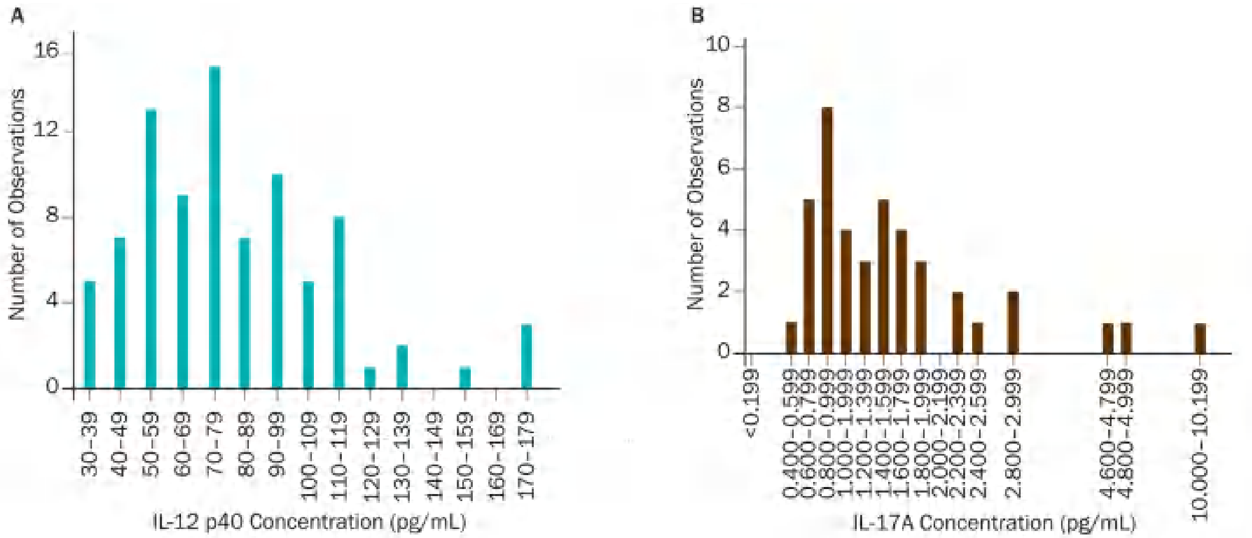
Two types of precision should be considered, intra-assay precision and inter-assay precision. Intra-assay precision is the reproducibility between wells within an assay. This allows the researcher to run multiple replicates of the same sample on one plate and obtain similar results. Inter-assay precision 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.



ReedBiotech 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. ReedBiotech Traditional conventions ELISAs are optimized to ensure high signal, low background, and the best sensitivity possible.



The Minimum Detectable Dose for Many ReedBiotech 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).

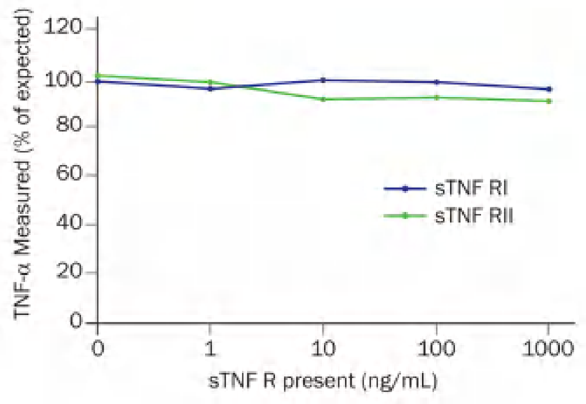
Linearity Experiments Identify False Positive Signals

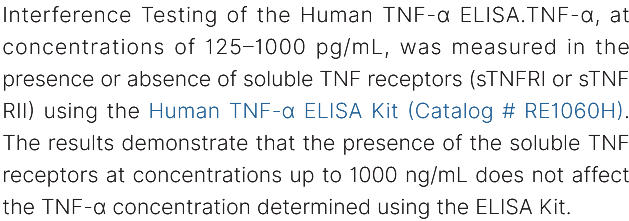
False Positive ELISA Signals Can Be Identified by Assaying the Linearity of Dilution.Serial dilutions of a cell culture supernate were assayed for natural linearity using two different MMP-9 ELISA Kits.

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.

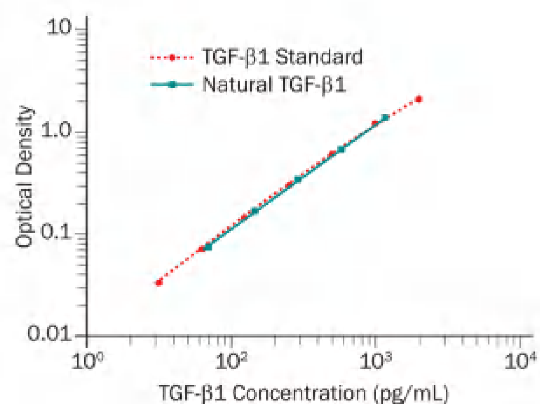
Sample Dilution	MMP-9 Kit	Competitor Kit
	Analyte Concentration Detected (ng/mL)*	
	5.26	19.63
1:2	104%	69%
1:4	106%	ND
1:8	103%	ND
Linearity claim	85–115%	69–128%

* 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 (sTNFRI 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.



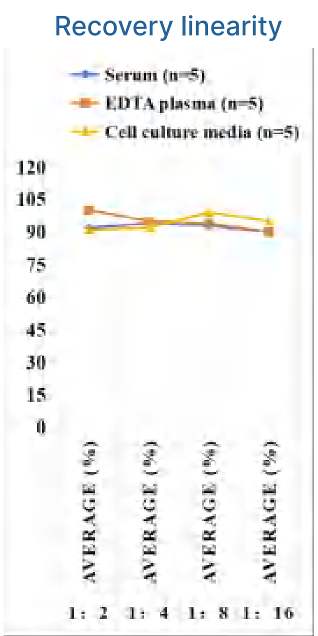
ReedBiotech Traditional conventions ELISA Kits Are Developed to Detect Natural and Recombinant Proteins. A serum sample containing activated human TGF-β1 was serially diluted (blue line) and compared to the TGF-β1 standard curve (red line). Results show that the Human TGF-β1 ELISA Kit (Catalog # RE10013)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

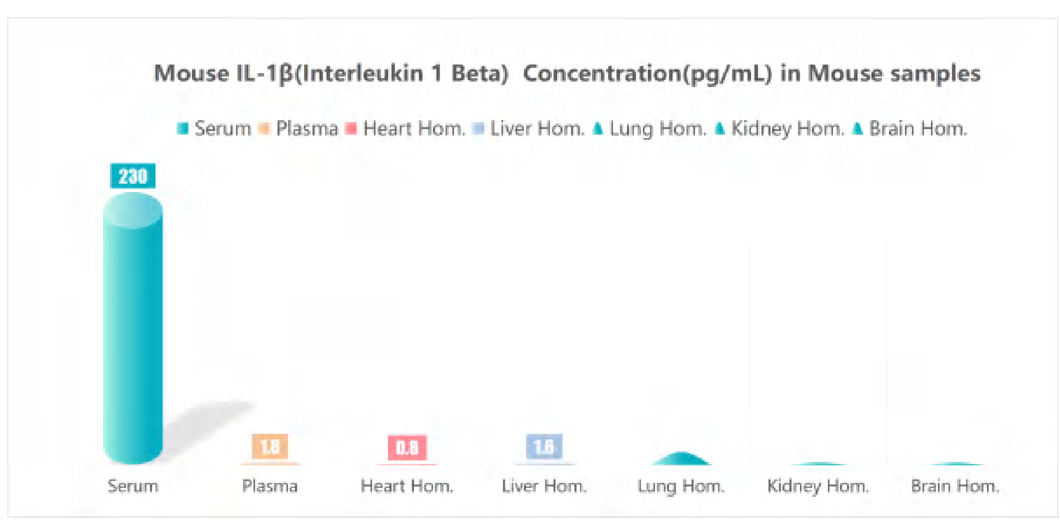
What is the importance of ELISA controls?

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.



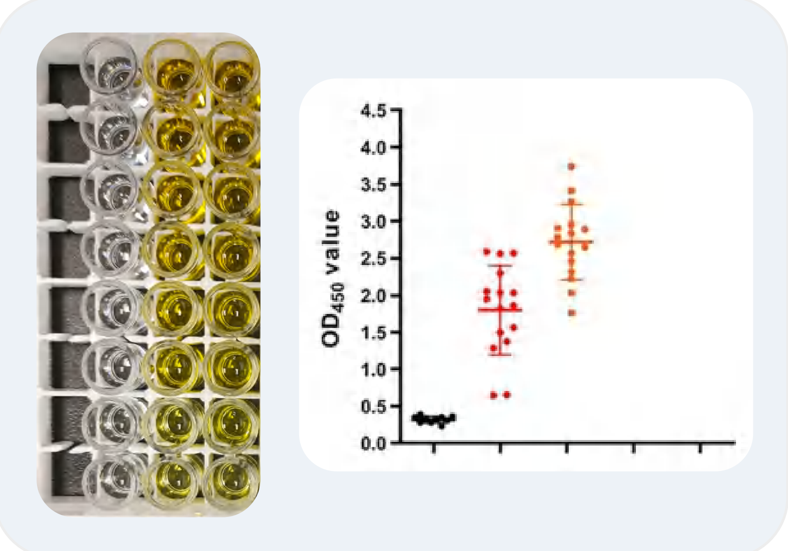


Blank Controls

The assay diluent is used as a blank sample to measure background absorbance and to zero the instrument baseline. Readings from test wells, negative controls, and positive controls should all be corrected by subtracting the blank background value before calculating the mean OD values for each group.

Negative Controls

A negative control is a sample known not to express the protein being tested. The purpose of using a negative control is to check for non-specific binding and false-positive results.



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 samples using blood collection tubes containing anticoagulants. Within 30 minutes after collection, centrifuge the samples at 1000 × g for 15 minutes at 2–8°C. Collect the supernatant for analysis.

Serum

Allow whole blood samples to stand at room temperature for 1 hour or overnight at 2–8°C. Then centrifuge at 1000 × g for 20 minutes at 2–8°C. Collect the supernatant for analysis.

Tissue Homogenates

Rinse the tissue with pre-cooled PBS (0.01 M, pH 7.4) to remove residual blood, weigh the tissue, and mince it into small pieces. Add the minced tissue to an appropriate volume of PBS (generally at a weight-to-volume ratio of 1:9; for example, 1 g tissue with 9 mL PBS. The exact volume may be adjusted according to experimental requirements and should be recorded). Addition of protease inhibitors to PBS is recommended.

Transfer the mixture into a glass homogenizer and thoroughly homogenize on ice. To further lyse tissue cells, the homogenate may be subjected to repeated freeze-thaw cycles or ultrasonic disruption. Finally, centrifuge the homogenate at 5000 × g for 5–10 minutes at 2–8°C, and collect the supernatant for analysis.

Cell Culture Supernatant

After collecting the culture medium, centrifuge at 1000 × g for 20 minutes at 2–8°C to remove impurities and cell debris. Collect the supernatant for analysis.

Precautions for Sample Collection

① Blood collection tubes should be disposable, pyrogen-free, and endotoxin-free. Whole blood samples should not be frozen directly; they must first be processed into serum or plasma before testing or storage.

② Common anticoagulants such as sodium or potassium EDTA and heparin salts may be used for plasma collection in ELISA experiments. However, for other assays such as biochemical tests, the effect of anticoagulants should be evaluated by the researcher.

③ Samples may be stored at 2–8°C if testing will be performed within one week after collection. If testing cannot be performed promptly, aliquot samples according to single-use volumes and store at: −20°C for testing within 1 month, or −80°C for testing within 3 months.

④ Avoid repeated freeze-thaw cycles. Before testing, frozen samples should be thawed slowly and centrifuged to remove precipitates generated during freeze-thawing. Mix thoroughly at room temperature before use.

⑤ Do not use lysis buffers containing surfactants (such as SDS) or organic reagents (such as methanol) for preparing tissue homogenates, cell extracts, or other sample types.Certain recombinant proteins may not be detectable because they are incompatible with the capture or detection antibodies used in the kit.

Sample Processing methods in special circumstances

Regarding high-fat samples: High-fat samples contain fat and are not homogeneous solutions, direct loading will affect the binding of antigen and antibody, resulting in inaccurate measurement results.It is recommended to centrifuge at a high speed of 5000×g first, let it stand for 10 minutes, take the clearer liquid in the middle layer, and then centrifuge it at a high speed (≥5000×g) to take the clear liquid in the middle and lower layers for detection.

Regarding hemolyzed samples: After the sample is hemolyzed, the endogenous HRP enzyme and the HRP-like properties of hemoglobin will cause uncontrollable nonspecific coloring in ELISA, affecting the accuracy of the test results.If the sample is slightly hemolyzed, the sample can be diluted and tested to reduce its impact or centrifuged to obtain the supernatant if the concentration range allows. If hemolysis is severe, it is recommended to re-collect samples.

Treatment of samples similar to cell supernatants

--Urine: Collect urine samples, usually the middle of morning urine, and add them to sterile tubes; Samples were centrifuged at 2-8 ° C, 1000×g, for 15-20 min to remove impurities and cell debris. Take the supernatant for detection.

--Saliva: Fasting 1 hour in advance and gargle to keep the mouth clean. Do not drink water or brush your teeth half an hour before sampling. At 2-8°C, centrifuge at 4000×g for 10 minutes to remove impurities, and take the supernatant to detect. A fresh saliva sample is recommended.

--Bone marrow fluid: It is recommended to use anticoagulant tube for anticoagulation and rest, centrifuge at 2-8°C, 1000×g for 30min for 15 min, and take the supernatant for detection. It is recommended that the bone marrow fluid be treated and tested directly after extraction. If it cannot be detected immediately, the supernatant after centrifugation should be frozen. Bone marrow is an important hematopoietic tissue, and the untreated samples will have hemolysis after direct freezing and thawing, so ELISA detection is not applicable.

--Alveolar lavage fluid: 10% chloral hydrate solution, abdominal anesthesia; open the abdominal cavity, take another 10mL syringe to collect blood from the inferior vena cava, and drain as much blood as possible. Lung lavage on the left lung on the ice. 5mL of normal saline at 37°C was taken and irrigated three times: 2mL, 1.5mL and 1.5mL. The recovery rate is more than 70%; The alveolar lavage solution was centrifuged at 4°C, 1000×g, for 10min, and the supernatant was detected.

--Joint fluid: Use a sterile syringe and needle to puncture the joint cavity and gently extract the joint fluid. The collected biological fluids were centrifuged at 4°C, 1000×g, for 20 minutes, and the supernatant was detected.

--Ascites: Puncture the abdominal cavity with a sterile syringe and needle to extract ascites. The collected biological fluids were centrifuged at 4°C, 1000×g, for 20 minutes, and the supernatant was detected.

--Cerebrospinal fluid: The subject is anesthetized, the spinal cord cavity or ventricle is punctured with a sterile needle, and the cerebrospinal fluid is slowly extracted. Collect in sterile containers. The collected biological fluids were centrifuged at 4°C, 1000×g, for 20 minutes, and the supernatant was detected.

--Nasal lavage solution: Use PBS(0.01M,pH=7.4) to inject the solution into the nasal cavity through a sterile syringe. The nasal lavage solution is recovered through the nasal cavity and collected in a sterile container. The collected biological fluids were centrifuged at 4°C, 1000×g, for 20 minutes, and the supernatant was detected.

The volume of lysis buffer must be determined according to the amount of tissue present. The typical final concentration of extracted protein should be >1 mg/mL.

Cell/tissue extraction buffer formulation

– 100 mM Tris，pH 7.4
– 150 mM NaCl
– 1 mM EGTA
– 1 mM EDTA
– 1% Triton X-100
– 0.5% Sodium deoxycholate

Other reagents required for complete extraction buffer preparation.

– Phosphatase inhibitor mixture
– Protease inhibitor mixture
– PMSF

Adding the phosphatase and protease inhibitor mixture to the cell extraction buffer according to the manufacturer's instructions before using,
The final concentration of PMSF was 1mM.

Data Analysis: Calculation Results

(An online calculation tool is available on the ReedBiotech website.)

Calculating Concentration of Target Protein in the Sample

1.When plotting the standard curve, first calculate the mean OD values of the standard and sample replicates, then subtract the OD value of the blank well to obtain the corrected value. Next, use concentration as the x-axis and corrected OD values as the y-axis, and perform nonlinear four-parameter fitting using appropriate software to generate the standard curve and calculate sample concentrations accordingly.

2.The final concentration of the sample is equal to the calculated sample concentration multiplied by the corresponding dilution factor.

(pg/mL)	O.D.	Average	Corrected	Standard Curve
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			

Calculation of Coefficient of Variation

Coefficient of Variation (CV) = SD / Average, usually expressed as a percentage. In general, the CV value between replicate wells should be within 20%.

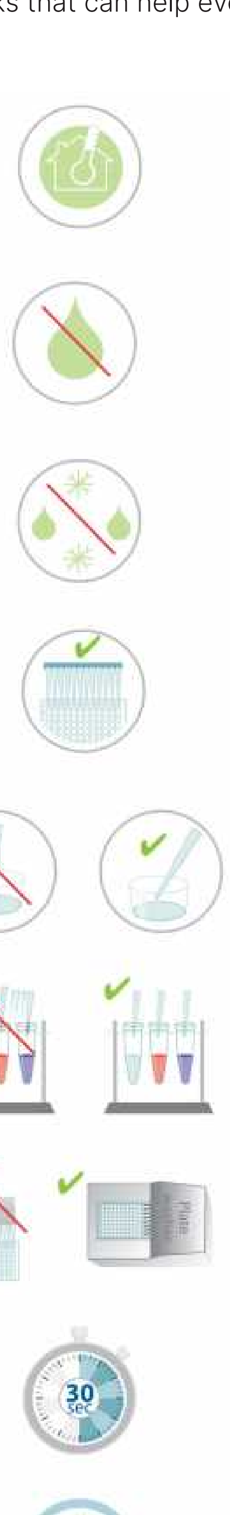
① first plot the standard curve. Next, treat standards as unknowns and interpolate the O.D. values from your standard curve. They should read close to the expected values(+/- 10%). Use the data reduction method that gives the best correlation value and backfit.

② 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.

③ A representative standard curve is shown in the figure below from Human High Sensitivity IL-4(Interleukin 4) ELISA Kit (Cat#RE1055HG).

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.

ReedBiotech ELISA Kit FAQs

1. How do I choose between different ELISA kit series?

Traditional ELISA Kits: Broad applicability with a wider range of targets.

Micro Fast ELISA Kits: Ideal for precious samples or samples with limited volume.

High Sensitive (HS) ELISA Kits: Recommended for samples with very low analyte concentrations.

Fast Step Series Kits: Suitable for rapid and high-throughput detection.

2. How should ELISA kits be stored?

Unopened kits should be stored at 2~8°C.

Different components of opened kits should be stored according to the conditions specified in the instruction manual.

3. How should samples be stored?

If testing can be performed within 1 week after sample collection, samples may be stored at 2–8°C. If testing cannot be performed promptly, aliquot the samples according to single-use volumes and store at:

-20°C for testing within 1 month

-80°C for testing within 3 months

Avoid repeated freeze-thaw cycles. Before testing, frozen samples should be thawed slowly and centrifuged to remove precipitates formed during freeze-thawing. Mix well at room temperature before use.

4. How should samples be transported?

To avoid repeated freeze-thaw cycles:Unfrozen liquid samples should be transported with ice packs.Frozen samples should be transported on dry ice.

5. Can reagents from different companies or different kit batches be mixed?

No. Significant differences may exist in assay procedures, reagent formulations, and reaction conditions among different companies or different kit batches. Therefore, mixing reagents is strongly discouraged.

6. Why can’t some transgenic or heterologously expressed samples be detected? What type of samples are recommended?

Factors such as expression systems, post-translational modifications, and purification processes may alter the conformation or structure of these samples, preventing antibody recognition. Such differences are minimal in natural samples; therefore, natural samples are recommended whenever possible.

7.Why does the OD value decrease as the standard concentration increases?

The two most common ELISA formats are sandwich ELISA and competitive ELISA.In sandwich ELISA, OD values are positively correlated with concentration.In competitive ELISA, OD values are negatively correlated with concentration.

8. How many samples can be tested with one ELISA kit?

A standard ELISA assay usually requires one standard curve (8 wells).Therefore, if all standards and samples are run in single wells, one 96T ELISA kit can test up to 88 samples (96–8= 88).

9. Can I use a previous standard curve or the standard curve provided in the manual?

No. A new standard curve must be generated for every ELISA experiment because variations may occur between operators and experimental runs.If I do not have enough strips left, can I reduce the number of standard wells? What is the minimum number required?Since ELISA standard curves are generally analyzed using a four-parameter logistic (4PL) fit, at least 5 standard points are required.

10.What should be used to dilute samples? Can I use my own dilution buffer?

Samples must be diluted using the sample dilution buffer provided with the kit. During kit development, the optimal dilution buffer formulation was specifically optimized to achieve the best assay performance. Using other dilution buffers may lead to unpredictable experimental results.

Troubleshooting your ReedBiotech® 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 strepta-vidin-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

VeriQuant™ Immunoassay Kit FAQs

1. Compared with the ELISA kits we have been using, what are the advantages of VeriQuant™ Immunoassay Kits? In which scenarios is it worthwhile to switch?

VeriQuant™ Immunoassay Kits are designed to support all of the following applications simultaneously:

High-sensitivity detection of low-abundance targets

Precious or limited-volume samples

Wide dynamic range for samples with large concentration differences

Applications requiring extremely high specificity

Multiplex detection

2. Is the operation of VeriQuant™ Immunoassay Kits complicated? Our laboratory mainly uses ELISA. Can operators learn quickly? Is additional training required?

To match users' existing workflows, ReedBiotech has maximized the ease of operation of VeriQuant™. One of its core advantages is its user-friendly workflow, making it highly suitable for laboratories already experienced in ELISA and with basic qPCR experience.

① Ready-to-use premixed design

Key reagents are pre-mixed in advance. Users do not need to perform antibody-oligonucleotide conjugation or prepare complicated qPCR reagents separately. Simply add samples and reagents according to the instructions, which significantly reduces operational steps and experimental variability.

② Highly compatible workflow

Compared with ELISA, the workflow is further simplified, requiring only a single incubation step with no washing procedures. The additional qPCR amplification step is straightforward and easy to understand. Personnel with qPCR experience can typically become proficient after 1–2 runs with the help of our detailed protocol.

3. How specific are VeriQuant™ Immunoassay Kits? How do you reduce false positives and cross-reactivity?

VeriQuant™ Immunoassay Kits incorporate triple-specificity technology, including:

Specific antibody pair recognition

Proprietary designed and synthesized oligo labels

Site-specific antibody labeling

These features significantly enhance assay sensitivity and specificity.

In addition, the fully liquid-phase, one-tube reaction system eliminates the need for coating, washing, and sample transfer steps, thereby minimizing non-specific adsorption as well as contamination introduced during transfer and washing procedures.

4. What special instruments are required for VeriQuant™ Immunoassay Kits? Our laboratory only has a standard ELISA microplate reader. Can we still use the kits?

The core instrument required for Reed Biotech VeriQuant™ ready-to-use kits is a qPCR instrument (a standard real-time fluorescence quantitative PCR system is sufficient). No microplate reader, fluorescence microscope, or other specialized instruments are required.

5. What sample types can VeriQuant™ Immunoassay Kits detect? Is the sample compatibility similar to ELISA?

The sample compatibility of Reed Biotech VeriQuant™ Immunoassay Kits is generally consistent with ELISA and is suitable for common sample types including:

Serum, Plasma, Cell culture supernatant, Tissue homogenates

No changes to existing sample preparation workflows are required. Users only need to avoid nuclease contamination to ensure compatibility with qPCR amplification.

6. What is the shelf life of VeriQuant™ Immunoassay Kits? Are the storage conditions the same as ELISA kits?

The shelf life of Reed Biotech PLA ready-to-use kits is 12 months from the manufacturing date.Storage and transportation conditions:Any unused reagents should also be kept at -20°C. Transportation should be carried out using low-temperature phase-change materials to maintain reagent activity, especially for qPCR enzyme reagents.

7. What additional consumables are required when using VeriQuant™ Immunoassay Kits?

① Standard nuclease-free and pyrogen-free consumables (pipette tips, EP tubes, etc.)

② Two 96-well PCR plates (PCR 8-strip tubes or individual PCR tubes may also be used)

③ 0.5-10μL, 2-20μL, 20-200μL, 200-1000μL High-precision pipettes, centrifuge tubes, and low-retention disposable tips:0.5-10μL, 2-20μL, 20-200μL, 200-1000μL

④ 37°C incubator

⑤ Double-distilled water or deionized water

⑥ Fluorescence qPCR sealing film (high-adhesion pressure-sensitive type) and sealing scraper

⑦ Two 25 mL reagent reservoirs

⑧ 96-well low-temperature metal cooling block

⑨ RNase-free 1.5 mL centrifuge tubes

⑩ Sterile 15 mL centrifuge tubes and a microplate centrifuge

VeriQuant™ Immunoassay Kit Troubleshooting

Observation	Possible cause	Recommended action
No Ct values in data file	qPCR software was not set up properly.	Make sure that all 96 wells are designated as unknown.
		Make sure that the parameters including FAM, ROX passive was set.
		Make sure the camera collected is at the last cycle point.
Poor standard curve (poor recovery)	Improper serial dilution	Verify that amounts of Assay Dilution Buffer and recombinant protein is correct for each well.
		Verify that the range of dilutions are within the recommended range in the kit protocol.
	Contamination from well to well.	Make sure that the plates are sealed tightly so that no spillage happens during mixing and plate centrifugation.
		Make sure to change pipette tips in between wells or samples.
Poor standard curve (high low end CV) High CV only at the low end of the curve but not the linear portion of the curve	The assay is at the limit of sensitivity.	Acceptable data is when the CV is less than 20%, so data cannot be reliable in this range.

Problem	Possible cause	Recommended action
Poor standard curve (high CV)	Pipetting issue.	Verify that the pipettes are calibrated.
		Make sure to use low retention filter tips, which are important for small volume pipetting.
		Make sure that bubbles are minimized when pipetting up and down.
		Use best practices with pipetting, i.e., pipette liquid onto the side of the plate, visual examination.
		Use multi-channel pipettes where possible.
		Ensure that each pipette tip is tightly secured with the visually correct amount of liquid.
		Do not do reverse pipetting with small volumes.
		If small volume such as 2 μL is a problem with consistency, try increasing the volume to 5 μL.
	Improper mixing.	Verify that the plate is sealed properly using a hand tool.
		When mixing, make sure it is thoroughly done (i.e., pipette up and down 10 times or striking the plate hard enough so that liquid goes from one side of the well to the other) at all steps when introducing new components.
		Make sure that centrifugation of the plate occurs after the mixing step to ensure all reagents come to the bottom of the plate.
	Evaporation when using small volumes.	Minimize any setup time by using the working plate or another method so that small volumes are not exposed for long periods of time.
	Insufficient replicates.	Run samples in triplicate so that outliers are more easily identified

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