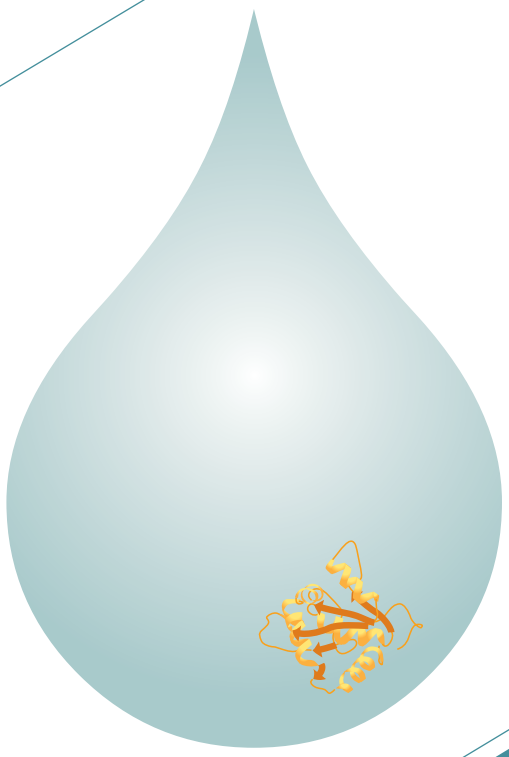
R&D SYSTEMS
a bio-techne brand

ELISA Guide



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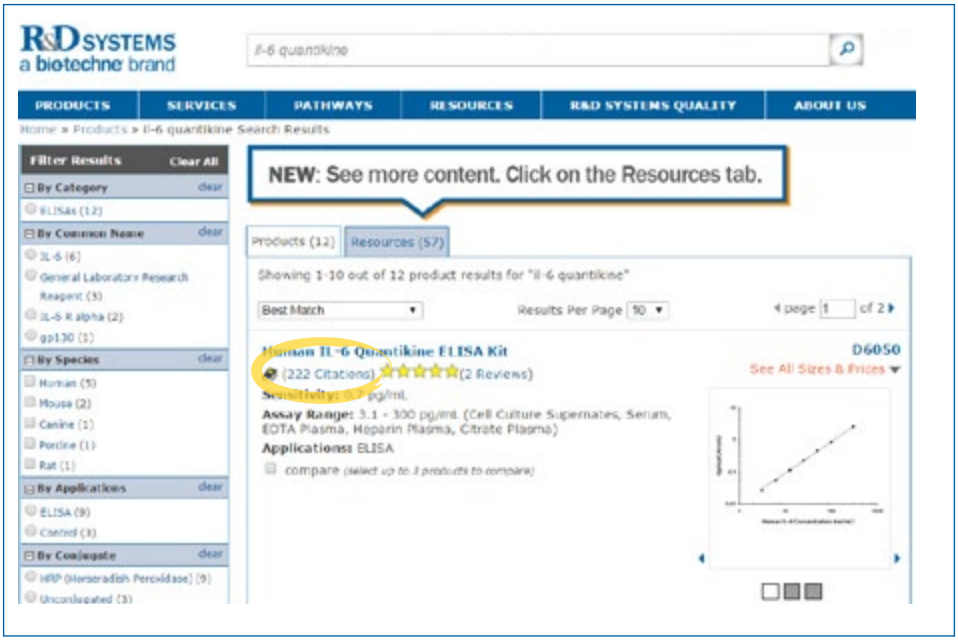
The Highest-Quality ELISAs Available

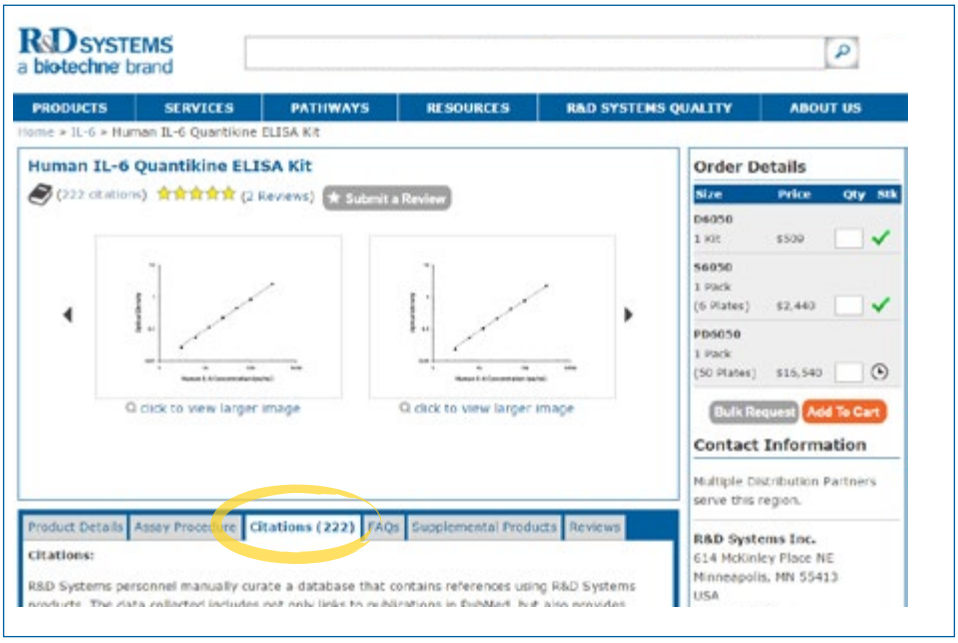
R&D Systems, a Bio-Techne brand, has over 25 years of experience designing, testing, and optimizing immunoassay kits to ensure the highest level of performance in analyte quantification. We currently offer more than 600 complete, ready-to-use Quantikine® ELISA Kits and 1,000 DuoSet® ELISA Development Systems for numerous different analytes and species, including human, mouse, rat, canine, primate, and porcine. Choosing quality reagents that will lead to results you can trust is one of the most critical aspects of scientific research. How do you know if you are choosing a quality product? One measure of product quality is the frequency of citations in the scientific literature. R&D Systems ELISAs are referenced more than any other ELISA manufacturer and we are honored by the trust that so many have placed in our products.

Find Citations on our Website

In seach results



On product web pages



Quantikine® ELISA Kits

Features and Benefits

R&D Systems offers a variety of Quantikine® ELISA kits that have been exhaustively tested for superior quality and reproducibility.

Ready-To-Use

Containing all of the supplies and reagents needed to perform an ELISA, Quantikine ELISA Kits are a complete solution for measuring target analyte concentrations.

Superior Performance

Carefully selected antibody pairs, precision automated microplate coating, and optimally formulated diluents ensure accurate results.

Rigorous Validation Testing

Validation testing for all Quantikine ELISA Kits includes intra- and inter-assay precision, assay linearity and recovery, as well as kit stability and assay drift. The detection of natural analyte is assessed in validated sample types and values are compared to published analyte concentrations.

Extensive Analyte Selection

R&D Systems offers over 600 Quantikine ELISA Kits with a selection of analytes that support a range of biological processes, including:

- cytokine and growth factor biology
- inflammation
- cancer biology
- angiogenesis
- apoptosis
- neuroscience
- nephrology
- cardiovascular biology
- obesity/diabetes
- bone metabolism

R&D Systems Quantikine ELISA Kits contain all of the components required to perform an immunoassay to measure the concentrations of natural or recombinant analytes.

Kit Components

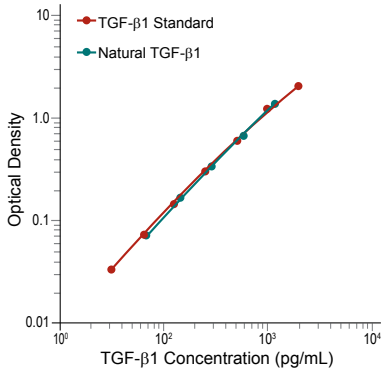
- Pre-coated 96-well Microplate
- HRP-conjugated Detection Antibody
- Mass-calibrated Standard
- Optimized Assay and Calibrator Diluents
- Wash Buffer
- Color Reagent A and B
- Stop Solution
- Plate Sealers
- Detailed Protocol

Ensuring Outstanding Performance and Consistency

The key to our ELISA quality is in-house manufacturing that allows us to conduct extensive validation testing.

Accurate Detection of Natural Proteins

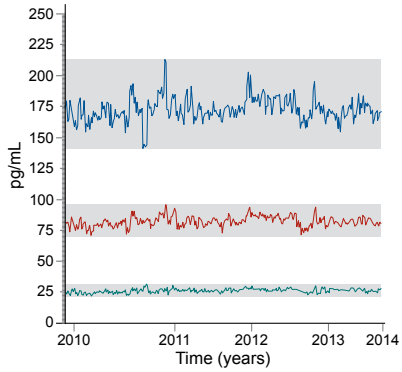
Antibody pairs recognize the supplied recombinant standard and the natural proteins in biological samples in a parallel manner, confirming that Quantikine ELISA Kits can be used to measure the relative mass values of the natural analyte. Protein standards are correlated to the NIBSC/WHO standard when available. R&D Systems has determined the ideal standard curve range for each assay, ensuring peak sensitivity and reproducibility of results.



Recognition of Recombinant and Natural Human TGF-β1. Serial dilutions of rhTGF-β1 standard (red line) or natural TGF-β1 present in serum (green line) were quantitated using the Human TGF-β1 Quantikine ELISA Kit (Catalog # DB100B). Quantikine ELISAs detect both recombinant and natural proteins in a parallel manner across a range of concentrations.

Confirmed Lot-to-Lot Consistency

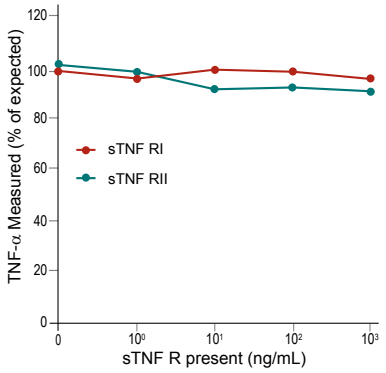
All lots are tested to ensure low background, a linear standard curve, consistent assay sensitivity, and a broad dynamic standard curve range. Consistent control values established during assay development ensure that sample data is comparable over time.



Quantitation of Human IL-6 in High, Medium, and Low Controls. High (blue line), medium (red line), and low (green line) controls are assayed with every manufactured lot of the Human IL-6 Quantikine Kit (Catalog # D6050). Controls for the Human IL-6 Quantikine ELISA Kit fall within acceptable ranges (gray bars) and remain constant from lot to lot.

High Specificity

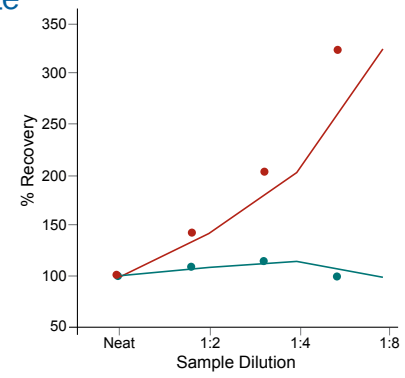
Every Quantikine ELISA Kit is extensively tested against related molecules to ensure no cross-reactivity or interference.



Quantikine ELISAs are Analyte-Specific. Concentrations of TNF-α ranging from 125-1,000 pg/mL were measured in the presence or absence of soluble TNF RI or soluble TNF RII using the Human TNF-α Quantikine ELISA Kit (Catalog # DTA00C). The presence of soluble TNF receptors did not affect TNF-α quantitation.

Precise Quantitation of Target Analyte

Assay diluents are carefully optimized for each Quantikine ELISA Kit in order to minimize assay interference due to non-specific binding, antibody interference, and cross-reactivity, which can be caused by assay surfaces or endogenous substances present in biological samples. Spike recovery and assay linearity experiments ensure that data are accurate for all validated sample types.



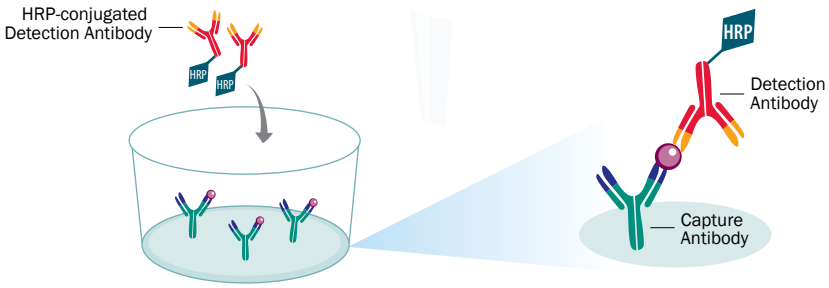
Assay Linearity as a Measure of Immunoassay Accuracy. Heparin plasma samples spiked with recombinant human Thrombomodulin were serially diluted. Thrombomodulin was quantitated using the Human Thrombomodulin/CD141 Quantikine ELISA Kit (Catalog # DTHBD0; green line) or an ELISA from a competitor (red line). Assay linearity values for the Quantikine ELISA kit fell within acceptable values (90–110% of the undiluted samples), while samples measured using a competitor's ELISA did not (141–325% of the undiluted samples).

Quantikine®—The Quality Story

When selecting an ELISA, different users often have different criteria in making their choice, whether it is sensitivity, sample types, or ease-of-use. One universal criterion is that an ELISA that must work the first time and every time you use it. R&D Systems has developed our Quantikine® ELISAs from raw materials that we make in-house, giving us unparalleled control over critical elements that affect your results and creating consistency of results over long periods of time. Whether you are breaking new ground or building on the work of other, you can trust that the results will be reproducible tomorrow, next week and next year.

Quality Antibodies Provide the Foundation for a High Performance ELISA

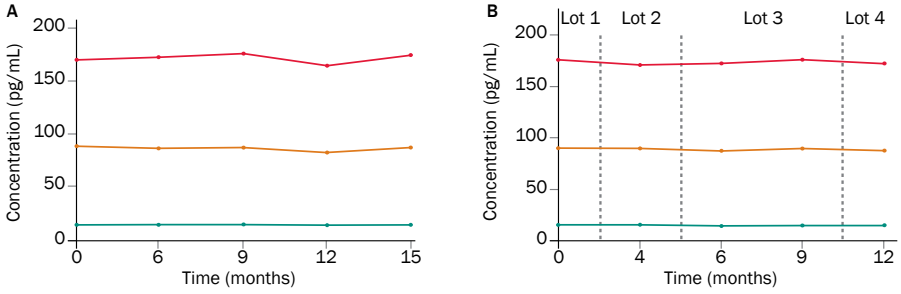
Our ELISA kits are developed using highly purified antibodies. For our sandwich ELISA kits, including the Quantikine, Quantikine HS, Quantikine IVD, and QuantiGlo Kits, testing is performed on several different monoclonal and polyclonal antibodies to determine which combination optimally couples for use in analyte detection. Selected antibodies are carefully titered to ensure that the concentrations chosen will give the best possible results for the assay. During development, the capture antibody is coated onto the microplate in several different concentrations to determine the concentration that offers the most binding and the best precision. Different concentrations of the detection antibody are subsequently tested to determine the concentration at which the detection antibody optimally pairs with the capture antibody to give the best signal-to-noise ratio.



Antibody Pairs Are Carefully Selected for Quantikine ELISA Development. Quantikine ELISAs are based on a two-site sandwich principle in which two highly specific antibodies are used to detect the target analyte. Quantikine Kits provide a 96-well microplate pre-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 that binds to a different epitope on the target analyte is used to complete the sandwich. A substrate solution is subsequently added to produce a signal that is proportional to the amount of analyte bound.

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. R&D Systems Quantikine 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.



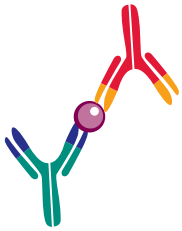
Quantikine ELISA Kits Are Tested for Stability and Reproducibility. A. Three samples with different concentrations of IL-6 (colored lines) were assayed using the same lot of the Human IL-6 Quantikine ELISA Kit (Catalog # D6050) over a 15 month period. **B.** Three samples with differing IL-6 concentrations (colored lines) were assayed using four different lots of the Human IL-6 Quantikine ELISA Kit (Catalog # D6050) over a 12 month period.

Specificity: Measuring Only the Analyte of Interest

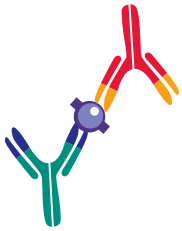
Immunoassay specificity can be compromised by antibody cross-reactivity and interference. Cross-reactivity occurs when a molecule other than the analyte of interest is bound by both antibodies leading to a false positive result. Interference occurs when other substances in the sample matrix modify the antigen-antibody interaction, preventing an assay from recognizing its designated analyte. These problems are mitigated by proper development and testing. False positive results may be due to matrix effects that only diligent validation and quality control measures can identify. This being the case, an ELISA can not be judged based solely on whether or not it produces a signal, until that signal is confirmed to be produced by the analyte of interest. In most cases, this can be accomplished by assaying the linearity of dilution (see previous page).

R&D Systems carefully selects antibodies, optimizes coating and conjugate buffers, and selects assay diluents to eliminate matrix effects. To gauge the specificity of an assay, factors related to the analyte are tested for cross-reactivity and interference. The members of the panel and the results of this testing are reported in our product data sheets.

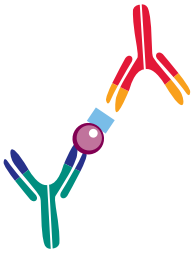
Binding differences may occur between natural and recombinant samples due to conformational changes of the antigen after it is bound to the capture antibody. These conformational changes may affect the binding of the detection antibody. Quantikine Kits are optimized so that the antibodies recognize both recombinant and natural antigen with equal efficacy.



Specific Interaction



Cross-reactivity



Interference

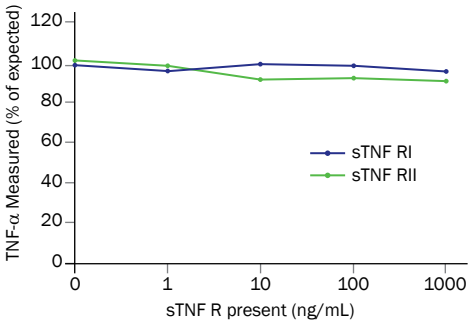
The Use of Quality Antibodies Is the First Step in the Development of a Reliable ELISA. Antibodies play a crucial role in the development of a high performance ELISA. Antibody pairs used for R&D Systems ELISAs are selected to ensure high signal, low background, and the best possible sensitivity. Antibodies are tested for specificity, cross-reactivity with molecules other than the target analyte, and interference with matrix components.

Linearity Experiments Identify False Positive Signals

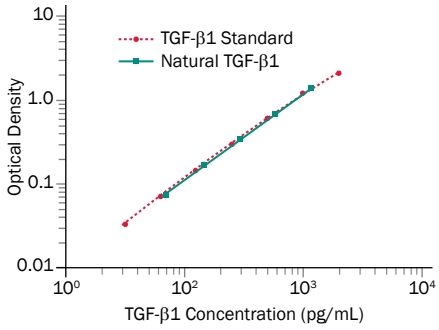
Sample Dilution	Quantikine Kit	Kit 2
	Analyte Concentration Detected (ng/mL)*	
	4.16	20.87
1:2	105%	73%
1:4	108%	ND
1:8	106%	ND
Linearity claim	85–115%	89–118%

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

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 TIMP-2 ELISA Kits. Diluted samples measured using the Human TIMP-2 Quantikine Kit (Catalog # DTM200) 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.



Interference Testing of the Human TNF-α Quantikine 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 Quantikine ELISA Kit (Catalog # DTA00C). 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 Quantikine ELISA Kit.



Quantikine 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 Quantikine ELISA Kit (Catalog # DB100B) measures recombinant and natural TGF-β1 with equal effectiveness.

Recovery

Complex sample matrices, such as serum and plasma, may contain interfering factors that affect the ability of an assay to accurately quantify the target analyte. Recovery experiments are used to determine if assays are affected by interfering factors. Low, medium, and high concentrations of analyte are spiked into all validated sample types and then analyzed for recovery. The results are expressed as a percentage of analyte recovered and are reported in each product data sheet. Our criteria require that recoveries are between 80–120% across the concentration range of the assay, demonstrating no quantifiable matrix interference for each sample type. If interfering factors are found, R&D Systems formulates diluents that minimize their effects.

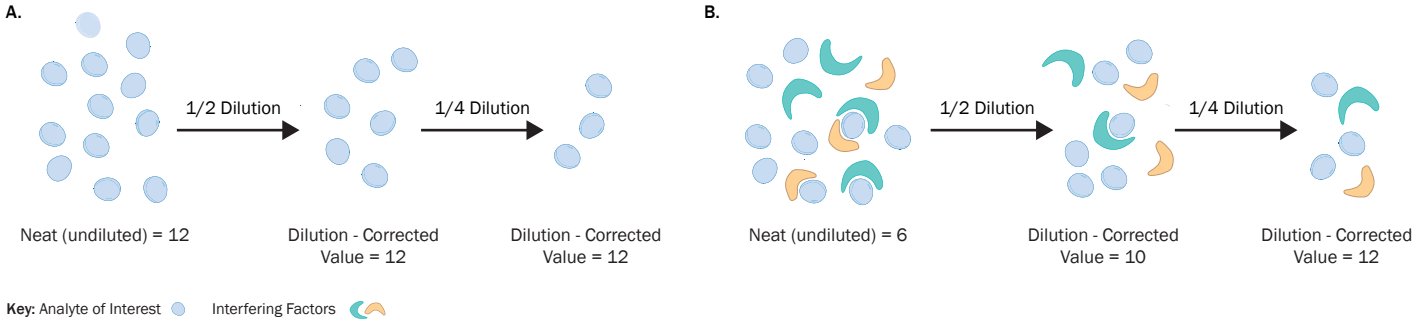
Dkk-1 Recovery Analysis

Sample	Average % Recovery	Range
Cell culture media* (n=4)	103	97–109%
Serum* (n=4)	99	85–115%
EDTA plasma* (n=4)	94	86–102%
Heparin plasma* (n=4)	98	92–106%
* Samples were diluted prior to the assay as directed in the product data sheet.		

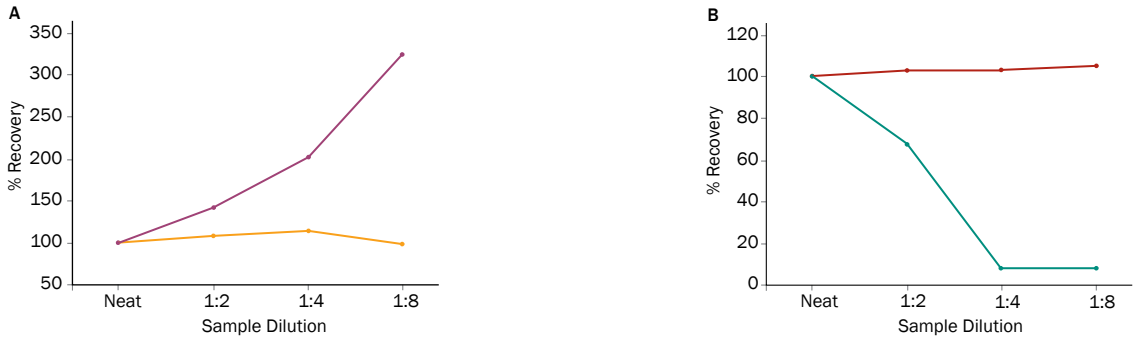
Analysis of the Recovery of Dkk-1 Using the Quantikine ELISA Kit. The recovery of Dkk-1 spiked to various levels throughout the range of the assay was assessed for all validated sample types of the Human Dkk-1 Quantikine ELISA (Catalog # DKK100).

Linearity of Dilution

Dilutions should always derive the same final analyte concentration for a sample. This is known as assay linearity. Interfering factors can compromise assay linearity unless the assay is designed to overcome these effects. We generate a dilution series using kit diluents across the dynamic range of the assay for each validated sample type. The results are expressed as a percent observed from expected. Values between 80–120% show good assay linearity. Each product data sheet documents the mean and range of percent linearity for all validated sample types.



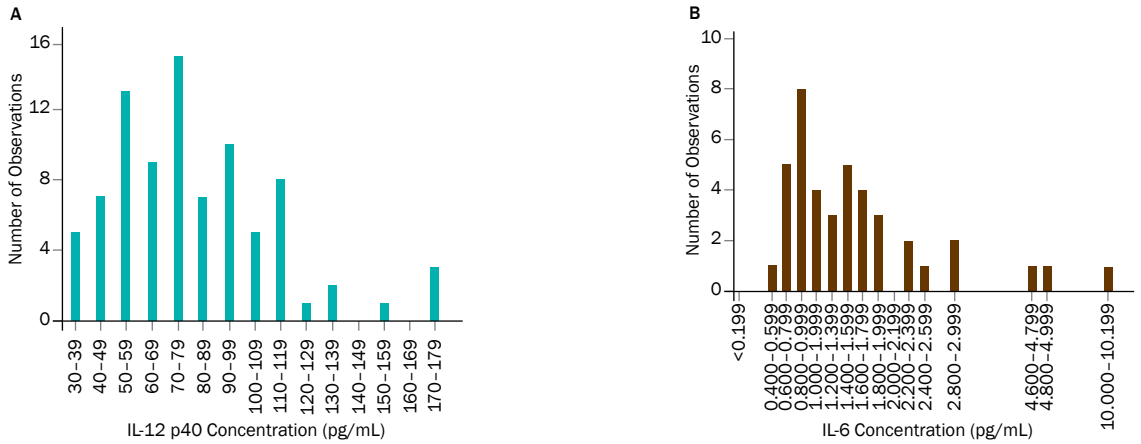
Linear Dilution to Assess Matrix Effects. A. Expected results from a linearity of dilution experiment when no interfering factors are present in the matrix. **B.** Potential results from the same experiment if interfering factors are present in the matrix. Factors in complex matrices can interfere with the analyte of interest. This effect may be revealed by unexpected linear dilution values.



Assay Linearity Is An Important Measure of Immunoassay Accuracy. A. Spiked heparin plasma samples were serially diluted and assayed for human Thrombomodulin using two different ELISA kits. Samples measured with the Human Thrombomodulin/BDCA-3 Quantikine ELISA Kit (Catalog # DTHBD0) had recovery values between 90–110% of the neat sample and displayed acceptable assay linearity (gold line). In contrast, the percent recovery of diluted samples measured with the second kit had a range of 141–325% (burgundy line), suggesting that interfering factors were preventing accurate measurement of the target analyte. **B.** Spiked heparin plasma samples were serially diluted and assayed for human Tissue Factor Pathway Inhibitor (TFPI) to determine the accuracy of two different immunoassay kits. Samples measured with the Human TFPI Quantikine ELISA Kit (Catalog # DTFP10) showed acceptable recovery values (90–110% of the neat sample; red line), while those measured with the second kit (green line) did not (80–7% of the neat sample).

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



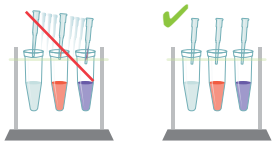
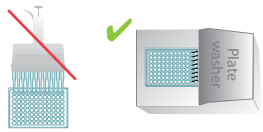
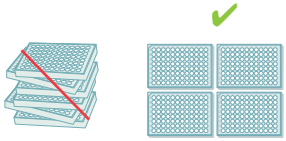
The Minimum Detectable Dose for Many Quantikine ELISA Kits Allows Proteins Present at the pg/mL Range to be Accurately Measured. A. Serum from 86 apparently healthy individuals was assayed using the Human IL-12/IL-23 p40 Quantikine ELISA Kit (Catalog # DP400). **B.** Serum from 41 apparently healthy individuals was assayed using the Human IL-6 Quantikine HS ELISA Kit (Catalog # HS600B).

Calibration: Ensuring Consistency from One Lot to the Next

Each Quantikine® ELISA Kit includes an immunoassay standard that is calibrated against highly purified material. R&D Systems assigns a mass value to a standard based on comparison to a master calibrator. These master calibrators are manufactured during the development of an ELISA and are used to maintain the consistency of kit standards. All future lots are compared to the master calibrator to ensure that no drift in sample values occurs. Due to the fact that different mass value assignments are made for ELISA standards, sample values produced using one manufacturer's kit may not be directly comparable to those obtained using another manufacturer's kit. R&D Systems supplies a correlation to a WHO international reference material, when available. This calibration allows a researcher to take the values obtained with a Quantikine® ELISA Kit and compare them to values obtained with other assays (assuming that the other ELISA manufacturer provides this conversion factor as well).

Best Practices and Techniques

While R&D Systems builds Quantikine® 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.

Troubleshooting your Quantikine® 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

Quantikine® ELISA FAQs

What is included in a Quantikine® Kit?

Quantikine® 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 Quantikine® kit?

Most Quantikine® 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 R&D Systems Quantikine® 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 "Citations" 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?

Unfortunately, R&D Systems has not 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 R&D Systems. For a more detailed spike and recovery protocol, please contact Technical Service. **Note:** *Acceptable ranges should be determined individually by each laboratory. Please see the Citations tab for peer-reviewed papers utilizing a wide range of sample types.*

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)?

R&D Systems 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 R&D Systems 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 RD1 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. R&D Systems 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?

R&D Systems offers tri-level control sets for the Human Quantikine ELISA Kits (colorimetric), Quantikine HS ELISA kits (high sensitivity), and QuantiGlo® ELISA kits (chemiluminescent). 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, R&D Systems 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 R&D Systems immunoassay kits with consistency between manufacturing lots. In general, we would expect +/- 25% recovery of the amount stated on the vial when using the Quantikine® 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.

Can a partial Quantikine® ELISA plate be used?

The Quantikine® 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?

R&D Systems 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. R&D Systems 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?

R&D Systems develops and QCs most of our Quantikine® 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.

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.

Biomarker Testing Service

History

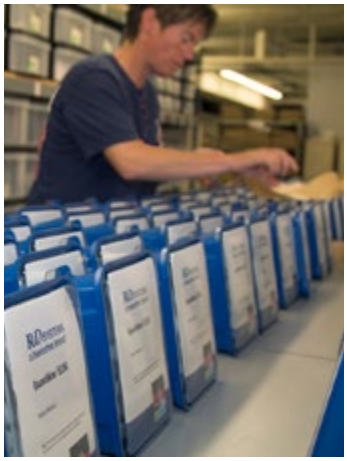
R&D Systems have over 20 years of experience designing, testing, and optimizing immunoassay kits to ensure the highest level of performance in analyte quantification. Support your preclinical and clinical studies with the same team that built your assay of choice.

Expertise

Scientists within the Biomarker Testing Service, a specialized division of R&D Systems, are specifically trained, operate under strict SOPs consistent with applicable GLP, and have full access to all R&D Systems products, technologies, and development expertise.

Delivery

Entrusting our expert personnel with your study samples ensures accurate results that are returned in a timely, efficient, and customized manner under our proven quality management systems.



Our Dedicated Team Specializes in the Following Sample Testing Services

- Biomarker testing utilizing state-of-the-art platforms:
  ELISA, Luminex®, Simple Plex™, Proteome Profiler™ Arrays, ELISpot, and Simple Western™
- Sample stability evaluation
- Sample management
- Sample collection and shipping kits
- Sample type validation

Why Use R&D Systems Biomarker Testing Service for your Research Study?

- **Expertise** Our Biomarker Testing Service scientists have full access to all R&D Systems products and development expertise.
- **Quality Assurance** Stringent quality assurance program evaluates all study activities, including test performance, control results, standard operating procedures, equipment, and personnel.
- **Trust** Our dedicated Biomarker Testing Service staff ensures your samples are treated in full accordance with the contracted study requirements. We provide rapid delivery of results and unsurpassed customer service.
- **Flexibility** The Biomarker Testing Service team has the flexibility to work on sponsor-directed studies to fulfill any specific requests for assay services.
- **Experience** Our Biomarker Testing Service laboratory successfully provides assay services for contract research organizations, academic groups, government organizations, and pharmaceutical companies.

DuoSet® ELISA Development Systems

Features and Benefits

DuoSet® ELISA Development Systems contain the essential components required to develop an immunoassay to measure the concentrations of natural or recombinant proteins.

Economical

Available with sufficient reagents for either five or fifteen 96-well plates, DuoSet ELISA Development Systems are a cost-effective solution for performing multiple ELISAs.

Optimized

Carefully selected and titrated capture antibodies and detection antibodies, a mass-calibrated standard, and a detailed protocol reduce your preliminary development requirements.

Flexible

Capture and detection antibody concentrations can be adapted to suit multiple platforms.

Versatile

Biotinylated detection antibodies and the streptavidin-HRP conjugate enable a choice of chemiluminescent or colorimetric substrates.

Extensive Analyte Selection

R&D Systems offers over 1,000 DuoSet ELISA Development Systems with a selection of analytes that support a range of biological processes, including:

- cytokine and growth factor biology
- inflammation
- cancer biology
- angiogenesis
- apoptosis
- neuroscience
- nephrology
- cardiovascular biology
- obesity/diabetes
- bone metabolism

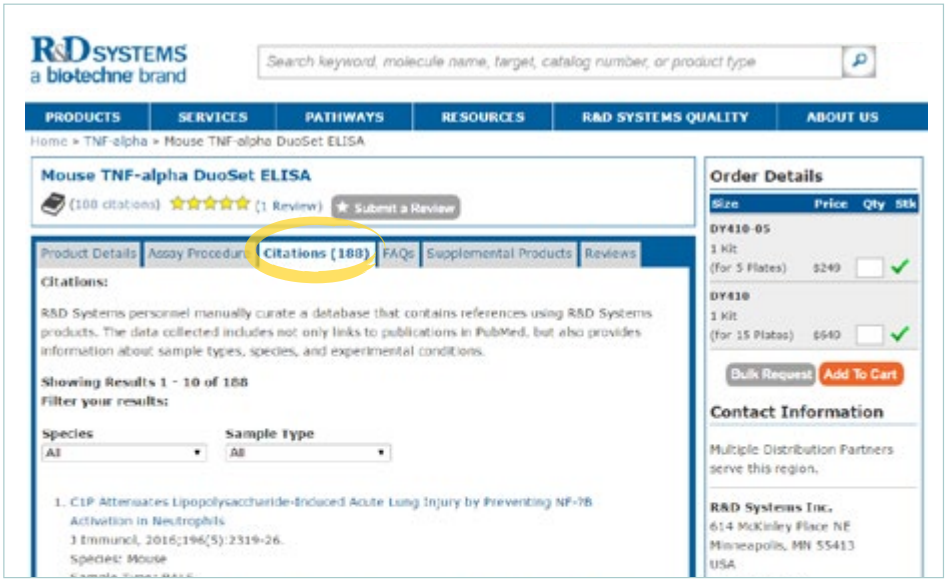
R&D Systems Duoset ELISA Development Systems contain the essential components required to develop an immunoassay to measure the concentrations of natural or recombinant proteins.

Components

- ELISA capture reagent
- biotinylated detection ntibody
- streptavidin-HRP
- mass-calibrated standard
- detailed protocol

Highly Referenced

For nearly 20 years, DuoSet ELISA Development Systems have been referenced in research literature. With over 25,000 product citations, our database makes it easy to view relevant references citing the use of DuoSet ELISA Development Systems. To simplify product selection, citations in our database include information on sample type and a direct link to the article.

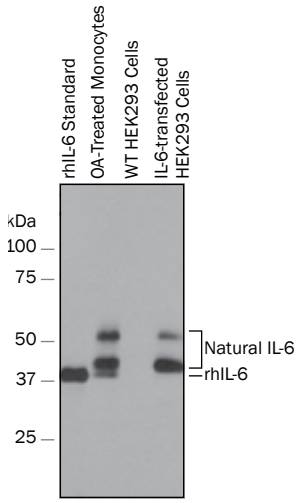


Ensuring Outstanding Quality and Performance

In-house manufacturing and stringent quality-control standards ensure the highest levels of performance and consistency.

Carefully Selected Antibody Pairs

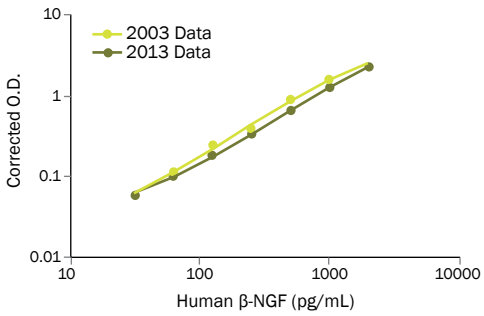
The capture and detection antibodies are carefully selected to ensure their flawless performance as a sandwich ELISA antibody pair. Antibodies are chosen for their specific recognition of target analytes and lack of non-specific analyte detection. Carefully titrated, the antibodies reflect the best concentrations for high top standard Optical Density (O.D.) and low background O.D. signals.



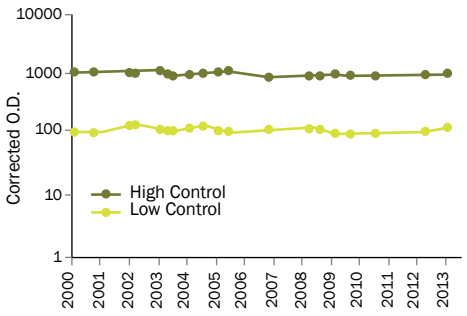
Specific Detection of Recombinant and Natural Human IL-6. The detection antibody from the Human IL-6 DuoSet ELISA Development System (Catalog # DY206) was used to detect recombinant human (rh) IL-6 standard (lane 1), in human monocytes treated with Okadaic Acid (OA; lane 2), wild-type (WT) HEK293 cells (lane 3), or *IL-6*-transfected HEK293 cells (lane 4) by Western blotting. The detection antibody specifically recognized recombinant, natural, and exogenously expressed human IL-6.

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 and control values ensure that sample data is comparable over time.



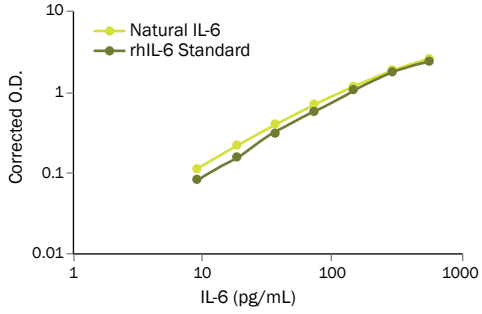
Comparison of Human β-NGF Standard Curve O.D.s from 2003 and 2013. Using the Human β-NGF DuoSet ELISA Development System (Catalog # DY256), standard curve values generated in 2003 and 2013 were compared for lot-to-lot consistency. Standard curve O.D.s remained consistent over ten years.



Quantitation of Human β-NGF in High and Low Controls. Controls are assayed with every manufactured lot of β-NGF DuoSet ELISA. Controls must read within a set range of ± two standard deviations from the mean. Controls for the β-NGF DuoSet ELISA Development System have remained consistent across 13 years.

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. R&D Systems 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 DuoSet ELISA Development System (Catalog # DY206). DuoSet ELISAs detect both recombinant and natural proteins in a parallel manner across a range of concentrations.

High Specificity

Assays are tested for cross-reactivity and interference with a panel of analyte-related molecules, ensuring their specific and accurate detection of the target analyte.

Assay Optimization

There are many parameters which influence the results obtained in an ELISA. These include: antibody quality and concentrations, incubation times, incubation temperatures, detection reagent quality and concentration, and substrate type and quality. For this section, it is assumed that all recommended reagents are being used.

Antibody concentration—the best way to determine the optimal capture and detection antibody concentrations is to perform a grid experiment. A grid experiment provides a method to test many antibody pair concentrations using only one plate. Antibody starting concentrations will vary depending on antibody type (monoclonal versus polyclonal) used for capture and detection, see Table 1. Refer to the product inserts for capture and detection antibody types as well as recommended starting concentrations.

Table 1. Recommended antibody starting concentrations

	Monoclonal Capture/ Polyclonal Detection	Monoclonal Capture/ Monoclonal Detection	Polyclonal Capture/ Polyclonal Detection
Capture Concentration	1, 2, 4 and 8 µg/mL	0.5, 1, 2 and 4 µg/mL	0.2, 0.4 and 0.8 µg/mL
Detection Concentration	50, 100, 200 and 400 ng/mL	0.25, 0.5, 1 and 2 µg/mL	50, 100, 200 and 400 ng/mL

To form the grid, divide a 96-well plate into 4 quadrants. See Figure 2 for an example of a monoclonal capture-polyclonal detection grid experiment. The 6 columns in each quadrant represent capture antibody concentrations, the 4 rows in each quadrant represent standard curve points, and each of the 4 quadrants represents a different detection antibody concentration. Each quadrant is a "mini-grid", identifying different capture antibody and standard concentrations at one particular detection antibody concentration. In the grid experiment in Figure 2, each quadrant contains all the possible combinations of capture antibody at 1, 2 and 4 µg/mL and standard curve points of ø (Diluent stated on the product insert), 1000, 2000, and 4000 pg/mL, at one detection antibody concentration.

From the multiple combinations of antibody pair concentrations illustrated on the grid, select the concentrations that give the best signal to noise ratio. The ø standard points give the "noise" or the background value that can be expected at each of the antibody pair concentrations. The 1000, 2000 and 4000 pg/mL standard curve points give the "signal" resulting from each of the many antibody pair concentrations. Select the highest signal to noise ratio that still gives an acceptable background. A signal to noise ratio of at least 10 is excellent, but the ratio should be at least five.

Figure 2. Grid experiment for monoclonal capture-polyclonal detection assay

	50 ng/mL detection						100 ng/mL detection					
	1	2	3	4	5	6	7	8	9	10	11	12
	1 µg/mL capture	**1 µg/mL capture**	**2 µg/mL capture**	**2 µg/mL capture**	**4 µg/mL capture**	**4 µg/mL capture**	**1 µg/mL capture**	**1 µg/mL capture**	**2 µg/mL capture**	**2 µg/mL capture**	**4 µg/mL capture**	**4 µg/mL capture**
A	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø
B	1000 pg/mL standard	1000	1000	1000	1000	1000	1000 pg/mL standard	1000	1000	1000	1000	1000
C	2000 pg/mL standard	2000	2000	2000	2000	2000	2000 pg/mL standard	2000	2000	2000	2000	2000
D	4000 pg/mL standard	4000	4000	4000	4000	4000	4000 pg/mL standard	4000	4000	4000	4000	4000
E	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø	ø
F	1000 pg/mL standard	1000	1000	1000	1000	1000	1000 pg/mL standard	1000	1000	1000	1000	1000
G	2000 pg/mL standard	2000	2000	2000	2000	2000	2000 pg/mL standard	2000	2000	2000	2000	2000
H	4000 pg/mL standard	4000	4000	4000	4000	4000	4000 pg/mL standard	4000	4000	4000	4000	4000
	200 ng/mL detection						400 ng/mL detection					

Background <0.2 O.D. units. Factors that influence background include: blocking reagent, capture and detection antibody concentrations, detection system, incubation times, diluents and washing technique.

Curve height preferably above 1.0, usually between 1.0 and 3.0 O.D. units. Factors that influence curve height include: capture and detection antibody concentrations (see grid experiment in Figure 2), incubation times and temperatures, detection system concentration, avidity of antibodies for antigens, pH, diluents and quality of reader.

Detection system assay sensitivity may increase with increasing detection reagent concentration or alternate detection system. However, this may result in higher background readings.

Dilution of serum and plasma samples serum and plasma samples may require a dilution of at least 2-fold in an appropriate buffer to overcome matrix effects. Empirically determine the dilution of the samples required to result in linearity of dilution. When diluting samples, remember that the diluent used for the standard curve should be the same as that used for samples. If samples are diluted, include the appropriate dilution factor when calculating results.

BSA bovine serum albumin, used as a blocking and carrier protein. Since different grades of BSA exist and may contribute to background, an ELISA grade BSA should be chosen and validated.

Incubation temperatures the sample and detection antibody incubations should be performed at room temperature. Sample incubation overnight at 4 °C or 1 hour at 37 °C may increase assay sensitivity, but may also increase the background.

Incubation times sensitivity may be increased with a longer incubation time at room temperature. Be aware that the top of the curve may flatten out and become unusable, limiting the assay range. Additionally, background may increase.

Interfering substances it is important to be aware of the possible presence of interfering substances such as heterophilic antibodies or rheumatoid factors. Please refer to The Immunoassay Handbook, edited by David Wild, Nature Publishing Group, copyright 2001, for suggestions on how to control for these substances.

Reagent reconstitution and storage conditions reconstitution and storage instructions provided with each reagent must be followed to ensure proper reagent perfomance.

Sample preparation and storage while not every analyte has the same stability within a given matrix, there are general precautions which should be followed. Samples that are not used immediately after preparation should be stored in single use aliquots at -70 °C. A -20 °C freezer may be acceptable, depending on analyte, if it is a manual defrost freezer. It is best if the samples contain carrier protein. Multiple freeze-thaw cycles should be avoided.

Samples/standard volume use of a larger sample/standard size (200 µL per well vs. 100 µL per well) may increase sensitivity.

Substrate substrates can vary. However, choosing an alternate substrate will require additional assay condition optimization. Some substrates require a longer incubation time to get the curve to a reasonable height. If the substrate is functioning as expected, sensitivity may be enhanced by increasing incubation time. Monitor the plate as it is developing to avoid excessively high backgrounds. Typically, the incubation time ranges from 10 to 30 minutes. Use the correct filters required to read the appropriate wavelength for the substrate chosen. This information is available from the substrate vendor.

Use of a shaker at room temperature may increase sensitivity. Shakers may be used for some or all of the incubation steps. Incubation times should be determined empirically.

Washing follow washing instructions given in the ELISA Protocol, page 4. Insufficient washing can result in high coefficients of variation (CVs), high background and poor results.

Sensitivity varies for each antibody pair. Sensitivity is defined by reliable discrimination from the zero standard. Factors which influence sensitivity include: capture and detection antibody concentrations (refer to the grid experiment shown in Figure 2), incubation times and temperatures, avidity of antibodies for antigens, sample/standard volumes, pH, diluents and wash buffer formulation. However, there is a limit to the sensitivity that can be achieved with each antibody pair.

Troubleshooting your DuoSet® ELISA

Problem	Possible Cause	Solution
High Background	Insufficient washing	• See washing procedure
		• Increase number of washes
		• Add a 30 second soak step in between washes
	Too much streptavidin-HRP or equivalent	• Check dilution, titrate if necessary
	Insufficient blocking	• Check blocking solution calculations
		• Increase blocking time
	BSA impurities	• Use high-quality BSA and consider evaluating a different preparation of BSA
	Incubation times too long	• Reduce incubation times
	Interfering substances in samples or standards	• Run appropriate controls
	Buffers contaminated	• Make fresh buffers
No signal	Reagents added in incorrect order, or incorrectly prepared	• Repeat assay
		• Check calculations and make new buffers, standards, etc.
	Contamination of HRP with azide	• Use fresh reagents
	Not enough antibody used	• Increase concentration
	Standard has gone bad (if there is a signal in the sample wells)	• Check that standard was handled according to directions
		• Use new vial
	Buffer containing FCS used to reconstitute antibodies	• Requalify your reagents of choice
	BSA impurities	• Use high-quality BSA and consider evaluating a different preparation of BSA
	Capture antibody did not bind to plate	• Use an ELISA plate (not a tissue culture plate)
		• Dilute in PBS without additional protein
	Buffers contaminated	• Make fresh buffers
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
	Too much streptavidin-HRP	• Check dilution, titrate if necessary
	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
	Buffers contaminated with metals or HRP	• Make fresh buffers
Standard curve achieved but poor discrimination between points (low or flat curve)	Not enough streptavidin-HRP	• Check dilution, titrate if necessary
	Capture antibody did not bind well to plate	• Use an ELISA plate (not a tissue culture plate)
		• Dilute in PBS without additional protein
	Not enough detection antibody	• Check dilution, titrate if necessary
	Plate not developed long enough	• Increase Substrate Solution incubation time
		• Use recommended time
	Incorrect procedure	• Go back to General ELISA Protocol; eliminate modifications, if any
	Improper calculation of standard curve dilutions	• Check calculations, make new standard curve
Poor Duplicates	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
	Uneven plate coating due to procedural error or poor plate quality (can bind unevenly)	• Dilute in PBS without additional protein
		• Check coating and blocking volumes, time and method of reagent addition. Check plate used
		• Use an ELISA plate (not a tissue culture plate)
	Plate sealer reused	• Use a fresh plate sealer for each step
	No plate sealers used	• Use plate sealers
	Buffers contaminated	• Make fresh buffers

Problem	Possible Cause	Solution
Poor assay to assay reproducibility	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	• Adhere to recommended incubation temperature
		• Avoid incubating plates in areas where environmental conditions vary
	Variations in protocol	• Adhere to the same protocol from run to run
	Plate sealers reused, resulting in presence of residual HRP which will turn TMB blue	• Use fresh plate sealer for each step
	Improper calculation of standard curve dilutions	• Check calculations, make new standard curve
		• Use internal controls
	Buffers contaminated	• Make fresh buffers
No signal when a signal is expected, but standard curve looks fine	No cytokine in sample or levels below assay range	• Use internal controls
		• Repeat experiment, reconsider experimental parameters
	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
Samples are reading too high, but standard curve looks fine	Samples contain cytokine levels above assay range	• Dilute samples and run again
Very low readings across the plate	Incorrect wavelengths	• Check filters/reader
	Insufficient development time	• Increase development time
	Coated plates are old and have gone bad	• Coat new plates
	Capture antibody did not bind to the plate	• Use an ELISA plate (not a tissue culture plate)
		• Dilute in PBS without additional protein
	Buffer containing FCS used to reconstitute antibodies	• Requalify your reagents of choice
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

DuoSet FAQs

How do I reconstitute my antibodies?

Please refer to the assay protocol or the lot-specific Certificate of Analysis. Assay inserts and CoAs can be downloaded from the product specific page on the R&D Systems website.

What samples can be tested in the DuoSet ELISA?

The diluent and assay protocol suggested by the DuoSet ELISA datasheet will be suitable for most cell culture supernatant samples. Sample types such as serum, plasma, and tissue homogenates need to be validated by the researcher prior to use in the DuoSet ELISA Development System. Spike/recovery and linearity experiments should be performed to determine whether sample values reported from unvalidated sample types are accurate. To obtain a copy of a suggested spike/recovery and linearity protocol, please contact our Technical Service department. It is beyond the scope of R&D Systems' intention to provide customers with advice in building reliable plasma and serum assays from DuoSet ELISAs. It is also not our intent to provide advice on how to QC and develop assays for sample types other than conditioned media. Customers wanting to measure protein levels in complex mixtures, like serum or plasma, should consult a reference guide, such as The Immunoassay Handbook, edited by David Wild, Nature Publishing Group, copyright 2001, or consider our Quantikine® ELISA kits.

Additional DuoSet FAQs can be found at rndsystems.com/duosetfaqs

Technical Service Troubleshooting Questionnaire

Please consult the Troubleshooting Guide to help resolve a problem before submitting this form to Technical Services
techsupport@bio-techne.com

Products Used

Plates

Manufacturer:_____ Catalog #: _____

Type: _____

Blocking Buffer

Buffer components: _____ BSA manufacturer:_____

Date of manufacture:_____ BSA catalog #: _____

Time/temperature:_____ BSA grade: _____

Substrate

Manufacturer:_____ Expiration date: _____

Catalog #: _____ Wavelength used:_____

Wash Buffer

Formulation: _____ Method of washing:

Number of washes: _____ _____ Wash bottle _____ Multi-channel pipette

Date of manufacture:_____ _____ Multi-channel manifold _____ Automated plate washer

Reconstitution of Reagents

	Capture Antibody	Detection Antibody	Standard
Catalog Number			
Lot Number			
Reconstitution Volume			
Reconstitution Buffer			
Reconstitution Date			
Storage after reconstitution			

Working Concentrations

	Capture Antibody	Detection Antibody	Standard	Secondary Reagent
Dilution Buffer				
Working Concentration				
Mixing Time				
Incubation Time				
Incubation Temperature				

Sample Type:

Were clean pipette tips and buffer reservoirs used at each step of the assay? _____

Did other assays performed on the same day, using the same substrate system work?_____

Was the protocol followed according to the assay insert? If not, what was done differently? _____

Summary of problem:

Please provide a copy of your labeled, raw (non-zero subtracted) O.D. data. _____

_____

Notes

Notes

Additional Product Lines

R&D Systems also offers a complete line of specialty ELISAs beyond our industry-leading Quantikine® and DuoSet® ELISAs. When your research requires high sensitivity, increased dynamic range, small molecule detection, or IVD clearance, there is a R&D Systems® kit to meet your goals, all with the high-quality antibodies and exhaustive testing you have come to expect.

Quantikine HS Colorimetric Sandwich ELISAs, High Sensitivity

Quantikine HS ELISA kits - greater sensitivity for samples containing very low levels of target molecule

Quantikine IVD Colorimetric ELISAs, for *In Vitro* Diagnostic Use

Quantikine IVD ELISA Kits - 510(k) cleared for *in vitro* diagnostic use

QuantiGlo Chemiluminescent Sandwich ELISAs

QuantiGlo ELISA Kits with broad dynamic range

Parameter Colorimetric Competitive ELISAs

Parameter Kits - competitive assays for cyclic nucleotides, eicosanoids, and more

Cell-Based ELISAs

Dual fluorescence detection of two biomolecules simultaneously in the context of a whole cell

DuoSet® IC (Intracellular) ELISA Development Systems

A format for measuring intracellular molecules in cell lysates by sandwich ELISA

DuoSet® IC (Intracellular) Phospho-specific ELISA Development Systems

A format for measuring phosphorylated intracellular molecules in cell lysates by sandwich ELISA

Supplemental ELISA/Assay Products

Microplates, wash buffers, diluents, detection reagents, stop solution, sample collection kits, and more

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