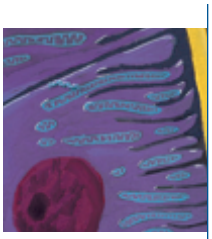


Proteins
Antibodies
ELISAs
Assay Services
MultiAnalyte Profiling
Activity Assays
Stem Cells
ELISpot Kits
Flow Cytometry
Cell Selection

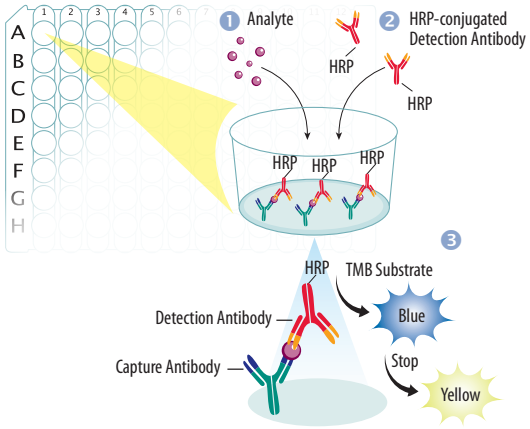
R&D Systems Quantikine® ELISA Kits

Quantikine Kits are complete, fully validated, ready-to-run sandwich ELISAs that are designed to provide the highest levels of specificity, accuracy, precision, and sensitivity in analyte quantification. Quantikine ELISAs are based on the two-site sandwich principle in which two highly specific antibodies are used to detect the target analyte.

Kit performance relies heavily on optimization during development including:

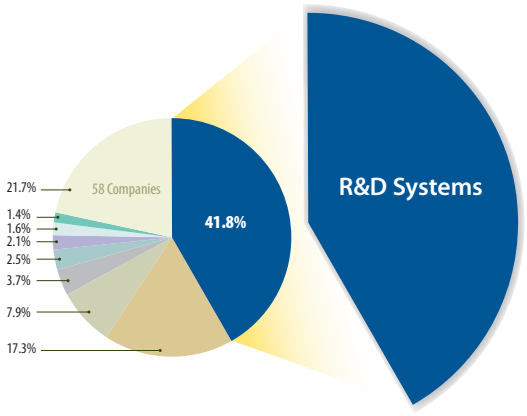
- Careful selection of antibody pairs
- Microplate coating with precision of less than 10% coefficient of variation (CV)
- Cross-reactivity and interference testing with a panel of up to 100 factors
- Special formulation of diluents to reduce interference due to matrix phenomena and/or heterophilic antibody interactions
- Correlation of the calibrated standard to NIBSC/WHO standards when available

Quantikine ELISA Assay Principle



A 96-well microplate pre-coated with capture antibody is included in every Quantikine ELISA kit. Experimental samples or standards are added to the wells and the target analyte binds to the immobilized capture antibodies (1). An HRP-conjugated detection antibody that binds to a different epitope on the target analyte is subsequently used to detect the bound protein (2). Tetramethylbenzidine (TMB) substrate solution is added to the wells and a blue color develops in proportion to the amount of analyte present in the sample. Color development is stopped turning the color in the wells to yellow. The absorbance of the color at 450 nm is measured, producing a signal that is proportional to the amount of analyte bound (3).

R&D Systems is the Most Referenced ELISA Manufacturer



A literature survey of 860 articles with citations that were published in 44 high impact journals from four different general research areas, including immunology, signal transduction, development/neuroscience, and bone/endocrinology/hematology, was conducted to determine the number of citations referencing R&D Systems ELISA Kits compared to the number referencing the use of ELISAs manufactured by other companies. The percentage of total citations attributed to several different ELISA manufacturers is shown. 433 total ELISA citations were identified in the survey, approximately 42% of these cited the use of R&D Systems ELISAs. The second largest section (21.7%) includes 94 citations referencing products from 58 different companies. No company included in this section had more than 1.2% of the total number of citations.

R&D Systems currently offers more than 300 Quantikine ELISA Kits for the quantification of proteins in a variety of sample types including serum, plasma, cell culture supernatants, cell lysates, saliva, human milk, urine, and more. These colorimetric assays allow for the measurement of:

- Adhesion Molecules
- Chemokines
- Cytokines
- Growth Factors
- Hematopoietic Factors
- Hormones
- Proteases

Kit Components*

- Antibody-coated 96-well Microplate
- Conjugated Detection Antibody
- Calibrated Immunoassay Standard
- Assay Diluent
- Calibrator Diluent(s)
- Wash Buffer
- Color Reagent A and B
- Stop Solution
- Plate Sealers
- Detailed Protocol

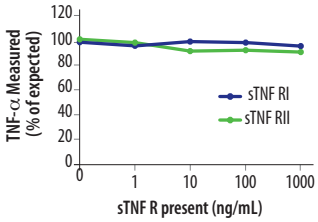
**Kit components may vary slightly. Please refer to the product-specific insert for a complete list of the contents for each individual assay.*

Evaluating Quantikine ELISA Kit Performance

Quantikine ELISA Kits undergo rigorous in-house testing to ensure optimal assay performance for all validated sample types. These tests are designed to assess the specificity, accuracy, sensitivity, and reproducibility of each immunoassay. By carefully considering the outcomes of the tests described below, scientists at R&D Systems take every step possible to ensure that Quantikine ELISA Kits will provide our customers with reliable, consistent results without the need for further assay optimization.

Specificity

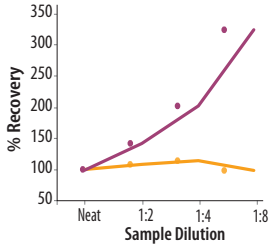
During the development of each Quantikine ELISA Kit, an extensive panel of factors related to the target analyte is tested to ensure minimal cross-reactivity and interference.



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 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.

Accuracy

Linearity of dilution: Quantikine ELISA Kits are tested with serial dilutions of a sample to ensure that each dilution derives the same end analyte concentration. Assays that fail to display linearity of dilution indicate that interfering factors are preventing accurate measurement of the target analyte. Proprietary technologies are utilized to develop diluents that alleviate any such interferences.



Assay Linearity Is An Important Measure of Immunoassay Accuracy. Heparin plasma samples spiked with recombinant human Thrombomodulin were serially diluted and assayed for recovery of the protein using two different ELISA kits. Samples measured with the Human Thrombomodulin/CD141 Quantikine ELISA Kit (Catalog # DTHBD0; gold line) showed acceptable recovery values (90-110% of the neat sample), while those measured with a second kit (burgundy line) did not (141-325% of the neat sample).

False positive results: Linearity of dilution experiments can also be used to determine if an assay is producing a false positive signal. False positive results may be due to matrix effects that only diligent validation and quality control measures can identify.

Sample Dilution	Quantikine	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 insert.

Linearity of Dilution Experiments Can Identify False Positive ELISA Signals. Serial dilutions of a cell culture supernatant were assayed for natural linearity using two different TIMP-2 ELISA Kits. Diluted samples measured with the Human TIMP-2 Quantikine ELISA Kit (Catalog # DTM200) gave recovery results of 105-108% of the neat sample, supporting the linearity claim. In contrast, the target analyte was undetectable beyond the first dilution in samples measured with a second kit, failing to support its linearity claim, and suggesting that the second kit was producing a false positive result. ND=Not detectable.

Recovery analysis: Quantikine ELISA Kits are assessed for analyte recovery in all validated sample types by assaying samples spiked with low, medium, and high analyte concentrations. Average recoveries must be between 90-110% across the concentration range of the assay. Recovery experiments, along with linearity of dilution analyses, determine if an assay is affected by interfering factors in the matrix.

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 insert.

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 Kit (Catalog # DKK100).

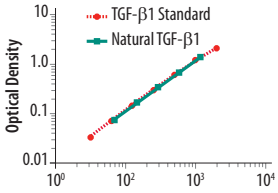
Precision & Reproducibility

Intra- and inter-assay precision: Results obtained with Quantikine ELISA Kits are confirmed to be reproducible from well-to-well and lot-to-lot.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	19.0	64.3	130	22.1	64.1	136
Standard deviation	0.7	2.4	4.5	1.6	5.4	9.3
CV (%)	3.4	3.7	3.5	7.2	8.4	6.8

Quantikine ELISA Kits Are Tested for Reproducibility. Intra-assay precision was assessed for the Human Clusterin Quantikine ELISA Kit (Catalog # DCLU00) by testing three samples of known concentration twenty times each on one assay plate (purple panel). Inter-assay precision for the same kit was determined by testing three samples of known concentration in forty separate assays (blue panel).

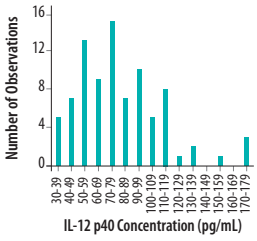
Analysis of natural samples: Quantikine ELISA Kits are developed to recognize both natural and recombinant antigen.



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 equally well.

Sensitivity

The minimum detectable dose for Quantikine ELISA Kits is determined by adding two standard deviations to the mean optical density value of several zero standard replicates and calculating the corresponding analyte concentration.



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

For research use only. Not for use in diagnostic procedures.

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