ELISA Guide

Your ELISA guidence
before your assay

CONTENT

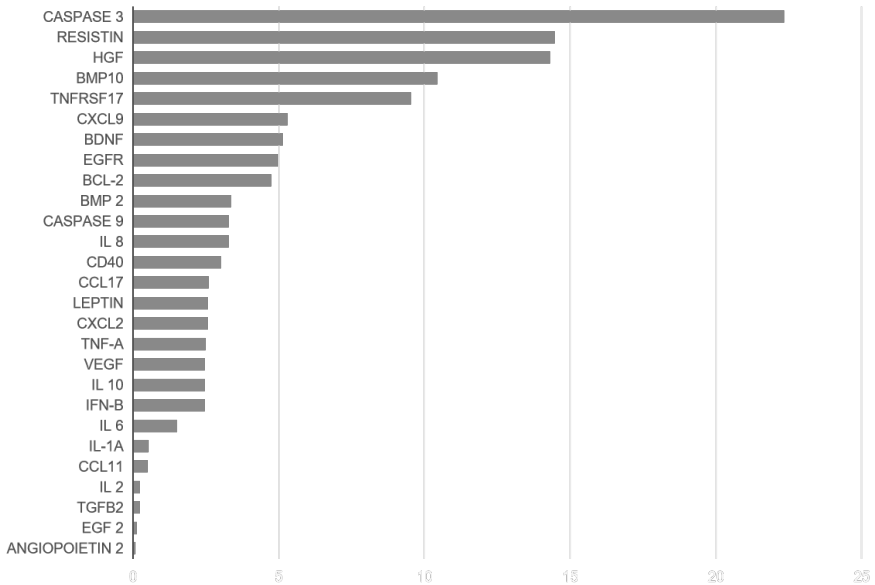
What's ELISA ?

The enzyme-linked immunosorbent assay (ELISA) is a commonly used analytical biochemistry assay. The assay uses a solid-phase type of enzyme immunoassay to detect the presence of a protein in a liquid sample using antibodies directed against the protein to be measured. In the most simple form of an ELISA, antigens from the sample (such as serum, plasma, cell culture supernates, cell lysates, saliva, tissue lysates, and urine) to be tested are attached to a surface. Then, a matching antibody is applied over the surface so it can bind the antigen. This antibody is linked to an enzyme and then any unbound antibodies are removed. In the final step, a substance containing the enzyme's substrate is added. If there was binding the subsequent reaction produces a detectable signal, most commonly a color change.

High sensitivity and specificity ELISA kit

Sensitivity of the assay is the smallest detectable amount of the target protein in question, it depends mainly on the affinity of the solid phase antibody according to the law of mass action. Specificity helps us recognize if the antibody detect the target protein specifically without cross-reacting with non-pecific proteins. Monoclonal antibodies have very high affinity for the antigen, which also have higher specificity than polyclonal antibodies. A monoclonal antibody is used as the detecting antibody in BT LAB sandwich ELISA to provide improved specificity.

- Extensive analyte selection
- Strict QC control
- Vadidated sample testing

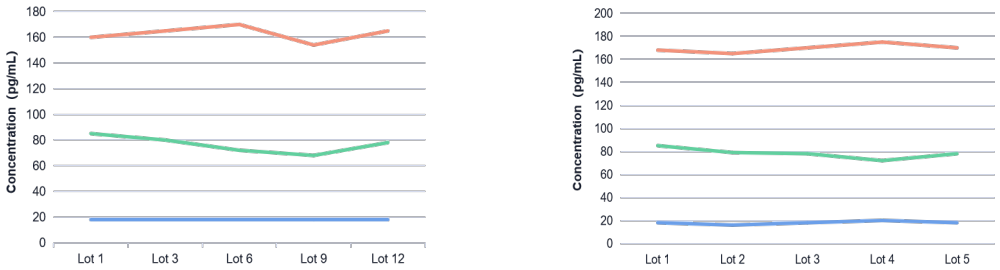


Superior sensitivity of BT LAB's popular Mouse kits

Precision & Reproducibility

Precision is measured as a coefficient of variation (CV) from the mean value.
CVs should be calculated from the calculated concentrations rather than the raw optical densities.

- Inter-assay % CVs of less than 15 are generally acceptable.
- Intra-assay % CVs should be less than 10.
- It is important that the CVs are reported for concentrations that reflect the range of results found in the specimens.



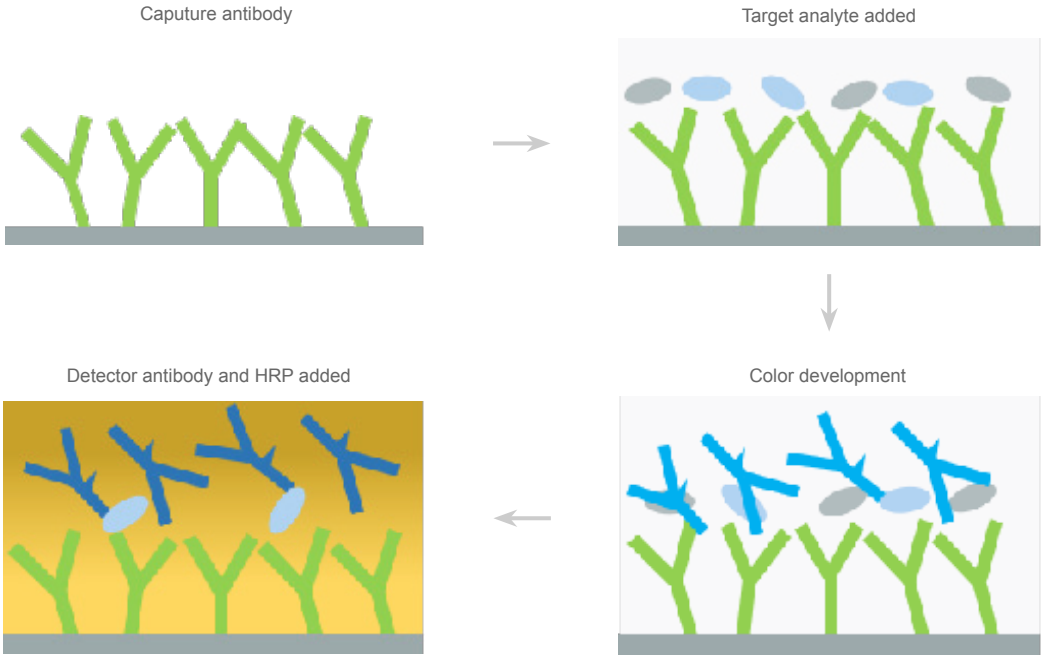
Different concentrations of various lots. BT LAB OneStep kits' CV values less than 10% across the standard curve for both intra- and inter-assay precision.

BT Lab ELISA Types

There are several ELISA configurations, with the most common formats being sandwich, competitive and signaling assays. Concentration-dependent detection of the target analyte is accompanied by either an increase or a decrease in signal, which is related to the format that is chosen.

Sandwich ELISA

In a sandwich ELISA, the goal is to use antibodies to precisely quantify specific antigen present in a solution (e.g., a serum protein). The primary antibody (also known as the 'capture' antibody) is added to all the wells of a microtiter plate and incubated to allow the primary antibody to adsorb to the surface of the well. Then a washing step ensure that only specifically bound molecules remain attached to the plate. A blocking protein is then added (e.g., albumin) to bind the remaining non-specific protein-binding sites in the well. Then samples of unknowns are added to each well and again allowed to incubate and bind to the capture antibody. After a washing step, the secondary antibody (also known as the 'detection' antibody) is added, which is a polyclonal antibody that is conjugated to an enzyme. After a final wash, a colorless substrate (chromogen) is added, and the enzyme converts it into a colored end product. The amount of color produced (measured as absorbance) is directly proportional to the amount of enzyme, which in turn is directly proportional to the captured antigen.

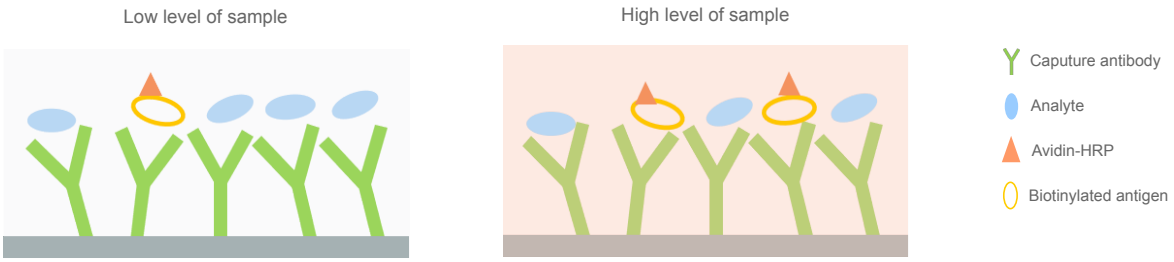


Featured product

Analytes	Code
IL-1 beta	E0001Gp E0143Hu E0192Mo
IL-4	E0036Bo E0003Ca E0052Ho E0092Hu E0051Mo E0120Po E0133Ra
IL-6	E0004Ca E0004Ch E0028Go E0090Hu E0049Mo E0122Po E0003Rb E0135Ra
IL-8	E0005Ca E0089Hu E0044Rb E0123Po E0327Ch E0073Sh E0052Cat
IFN-gamma	E0005Bo E0011Ca E0013Cat E0030Go E0105Hu E0056Mo E0100Po E0103Ra E0049Sh E0101Ho
TNF-alpha	E0019Ho E0082Hu E0117Mo E0299Po E0065Rb E0764Ra E0025Ca E0117Mo E0299Po E0065Rb E0764Ra E0025Ca
VEGF	E2102Bo E0019Ca E0080Hu E0114Mo E0269Po E0659Ra
IL-2	E0094Hu E0053Mo E0123Ra E0225Bo E0201Ca E0118Po E0134Rb
IL-10	E0102Hu E0022Mo E0108Ra
CRP	E0150Gp E0042Ha E1798Hu E0218Mo E0048Po E0016Rb E0053Ra
IFN-beta	E0154Hu E0195Mo E0095Ra
ACE2	E0130Bo E3169Hu E1226Mo E0968Ra
LEP	E1559Hu E0652Mo E0561Ra E0079Cat E0026Ch E0082Ca E0062Sh
TGF-beta1	E0041Ca E0066Gp E0134Hu E0660Mo E0304Po
BDNF	E0091Ho E0013Mo E0228Rb E0476Ra E2190Bo E6633Hu E0537Po
IFN-gamma	E0005Bo E0011Ca E0013Cat E0030Go E0105Hu E0056Mo E0100Po E0103Ra E0049Sh E0101Ho
CCL2/MCP-1	E0170Ho E4830Hu E1707Mo
MMP-9	E2279Bo E0357Ca E0936Hu E0277Mo E0321Ra
IGF-1	E0016Bo E0318Ch E0103Hu E0037Mo E0709Ra
IL-12	E3301Hu E0020Mo E1409Ra
GM-CSF	E0107Hu E0038Mo E0429Ra E0430Bo E0134Ca E0448Po

Competitive ELISA

In a competitive ELISA, the primary antibody is added to the sample which contains the antigen. The primary antibody will bind the antigen forming an antibody-antigen complex. The sample is then added to a plate which has antigen bound to each well. Primary antibodies which have already been bound to the antigen in the sample can not bind to the antigen on the plates. After a washing step, the more antigen in the sample the more primary antibody gets washed away. A secondary antibody is then added to the wells which binds the primary antibody. The secondary antibody is again bound to an enzyme which can catalyze a colormetric or chemiluminescent reaction. For competitive ELISA, a low signal from the enzyme means that there is high amount of antigen in the sample.



Featured product

Analytes	Code
CORT	EA0019Ch EA0056Rb EA0036Ra
GSH	EA0104Mo EA0142Hu EA0041Ca EA0037Fi EA0113Ra
PROG	EA0004Sh EA0005Go EA0008Bo EA0024Hu EA0016Mo EA0063Ra
COR	EA0021Bo EA0010Ge EA0007Go
Estradiol	EA0016Fi EA0030Hu EA0011Ra EA0018Sh
IgG	EA0025Go EA0000Gp EA0027Mo EA0033Ra
FSH	EA0015Ra EA0014Mo
GH	EA0006Bo EA0021Fi

Qualitative ELISA

ELISA may be run in a qualitative format. Qualitative results provide a simple positive or negative result (yes or no) for a sample. The cutoff between positive and negative is determined by the analyst and may be statistical. Two or three times the standard deviation (error inherent in a test) is often used to distinguish positive from negative samples. In quantitative ELISA, the optical density (OD) of the sample is compared to a standard curve, which is typically a serial dilution of a known-concentration solution of the target molecule. For example, if a test sample returns an OD of 1.0, the point on the standard curve that gave OD = 1.0 must be of the same analyte concentration as the sample.

Featured product

Analytes	Code
Brucella Ab IgG	ED0004Ca ED0470Hu ED0003Po ED0080Mo ED0000Go ED0000Sh ED0023Bo
HP Ab IgG	ED0022Hu ED0049Mo
HP Ab IgM	ED0023Hu
HEV-IgG	ED0034Hu ED0086Mo
RV Ag	ED0076Hu ED0018Po ED0039Mo ED0003Go ED0017Rb ED0002Ho ED0002Sh ED0018Bo ED0001Ca
PV Ab IgG	ED0022Ca
AQP-4 Ab	ED0546Hu
TB Ab IgG	ED0324Hu

Research Area

- Adipokines
- Angiogenesis Biomarkers
- Apolipoproteins
- Apoptosis Pathway Proteins
- Bone Metabolism Biomarkers
- Cancer Biomarkers
- Cardiovascular Disease
- Cell Signaling Pathway Proteins
- Cell Structure Proteins
- Chemokines/cytokines
- Growth Factors
- Immune checkpoint Proteins
- Immunoglobulins
- Kidney Injury Biomarkers
- Liver Proteins
- Metabolic Hormones
- Neurological Disorders Biomarkers
- Soluble Receptors
- Thyroid Hormones
- Vascular Injury Biomarkers

Sample Preparation

Serum Allow serum to clot for 1.5 - 2hrs at room temperature. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

Plasma Collect plasma using EDTA or heparin as an anticoagulant. After mix 10-20 minutes, centrifuge samples for 20 minutes at 2000-3000 RPM. Collect the supernatant without sediment.

Saliva Collect saliva using a collection device without any protein binding or filtering capabilities. Centrifuge at 10,000 x g and 4°C for 2 min. Assay immediately or aliquot supernatant and hold at -80°C. Avoid freeze/thaw cycles.

Cell culture supernatant Collect by sterile tubes. When detecting secrete components, centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatants. When detecting the components in the cell, use PBS (pH 7.2-7.4) to dilute cell suspension, the cell concentration of approximately 1 million/ml. Damage cells through repeated freeze-thaw cycles to let out the inside components. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

Tissue Rinse tissues in ice-cold PBS (pH 7.4) to remove excess blood thoroughly and weigh before homogenization. Mince tissues and homogenize them in PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. To further break down the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000 ×g to get the supernatant.

Urine Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge at 2000-3000 RPM for 20 minutes to to remove particulate matter. Assay immediately or aliquot and store at ≤ -20 °C.

Ascites/Cerebrospinal fluid Collect by sterile tube. Centrifuge at 2000-3000 RPM for 20 minutes. Collect the supernatant without sediment.

Tips for ELISA

- The instruction should be strictly followed in the experiment.
- All reagents should be brought to room temperature before use.
- Once the desired number of strips has been removed, immediately reseal the bag to protect the remain from deterioration. Cover all reagents when not in use.
- Make sure pipetting order and rate of addition from well-to-well when pipetting reagents.
- Pipette tips and plate sealer in hand should be clean and disposable to avoidcross-contamination.
- For standard 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.
- Do not allow wells to become dry during the assay procedure.
- When washing plates, either manually or with a plate washer, be sure to give the wash buffer time to work by soaking time.
- Stop solution contains acid. Please wear eye, hand and skin protection when using this material. Avoid contact of skin or mucous membranes with kit reagent.
- The kit should not be used beyond the expiration date.

ELISA FAQs

What is BT LAB's product use?

Products are sold for research or laboratory use only and are not to be administered to humans or used for medical diagnostics.

What is the shelf life of BT LAB's ELISA kit?

Most of our products have a guaranteed shelf life until their expiration date, when under proper storage and handling conditions as instructed on our product data sheets. For recombinant proteins, the minimum guaranteed shelf-life is 12 months from the date of receipt by the end-user.

Can plate in the ELISA kit be used partially?

BT LAB's ELISA plates have removable strips of wells. Unused wells can be removed from the plate and returned to the foil pouch, store it at 2-8°C for up to one month.

What sample can be tested in the kit?

Generally BT LAB kits are validated for serum, plasma and cell culture supernat. However, the samples validated in an ELISA can vary from different products. The product datasheet and product-specific web page states all sample types that have been validated for use with the ELISA kit. References may exist for other sample types.

Can I use the reagents in your ELISA kit with a protein standard from another company?

No, we do not recommend combining reagents from different ELISA kits or manufacturers. We cannot guarantee the performance of the kit if you combine reagents from different kits or manufacturers.

How many samples volume required with BT LAB's ELISA kit?

The kits require 40 uL.

Why I didn't get a signal in the sample but the standard curve looks good?

- Preparing the sample in the wrong way leads that the sample may not contain the analyte. A matrix effect interfere may be masking the detection.
- The sample or the antibody in the kit may be contaminated.
- Samples contain cytokine levels above assay range, you should choose the right kit with proper assay range.

What is sample matrix effect?

Matrix effects, or the effect that the other substances in your sample might have on the ability to detect your specific target protein, are most commonly observed when using plasma and serum samples. Matrix components can affect the binding of antibody to protein or alter the signal-to-noise ratio.

Can I cite BT Lab's product if I publish a paper?

Yes, if you publish a paper which cites BTLab's product as the source of one of your materials, please email us a copy at Word/PDF format of your paper for transparency and you will get reward.

Troubleshooting

High background

Possible case	Solution
Non-specific binding of antibody	Replace another purified antibody or blocking buffer
Plate are not be sealing incompletely	Make sure to follow the instruction strictly
Incorrect incubation temperature	Incubate at room temperature
Incubation time too long	Reduce incubation time
Substrate exposed to light prior to use	Keep substrate in a dark place
Substrate was contaminated	Replace substrate. Substrate should be clean and avoid crossed contamination by using the sealer
Contaminated wash buffer	Use a clean buffers and sterile filter
Improper washing	Increasing duration of soaking steps

Weak Signal

Possible case	Solution
A reagent or a step of the procedure omitted by mistake	Check protocol and follow steps carefully
Antibody are not enough	Increase the concentration of the antibody

Reagent are contaminated	Use new one
Pipette are not clean	Pipette should be clean
Wrong incubation time or temperature	Check and follow protocol recommendations. Place plates in an incubator during incubation periods to avoid temperature fluctuations (set to room temperature 25°C)
Improper washing	Increasing duration of soaking steps

No Signal

Possible case	Solution
Reagent are contaminated	Use new one
Sample prepared incorrectly	Review the process of sampling and sample preparation
Antibody are not enough	Increase the antibody concentration
Wash buffer contains sodium azide	Use a new wash buffer and avoid sodium azide in it
HRP was not added	Add HRP according to the instruction

Poor Precision

Possible case	Solution
Pipetting error	Dispense quickly and identically into the
Incomplete washing	Make sure wells are washed adequately by filling the wells with wash buffer and all residual antibody solutions crossed well before washing
Unclean wells	Inspect wells and remove debris prior to use. Wipe bottom of plate to remove any debris or fingerprints prior to reading

Poor standard curve

Possible case	Solution
Incorrect preparation of standard	Reconstitute standard as suggested on data sheet
Capture Antibody did not bind	Use BT LAB ELISA plate in the kit
Inefficient washing	Be sure wash apparatus is working properly (i.e. distributing even volumes into each well). Be sure wells are empty after aspiration, yet be sure to fill wells in a timely manner.
Pipetting error	Dispense quickly and identically into the side of each well. Use calibrated pipettes.
Incorrect storage of components	Store all components as recommended on data sheet. Do not allow reconstituted reagents to stay at room temperature for excess time.

Nots

Nots

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