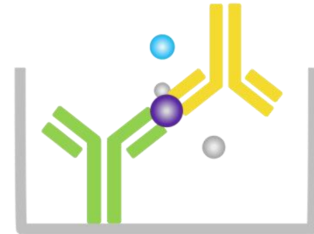
ELISA guide

Your ELISA guidance
before your assay

Catalog

What's ELISA? 2
ELISA Types 2
  Direct ELISA 2
  Indirect ELISA 2
  Sandwich ELISA 2
  Competitive ELISA 2
BT LAB ELISA portfolio 3
  Sandwich ELISA 3
  Competitive ELISA 4
  Qualitative ELISA 5
High sensitivity and specificity 6
Precision & Reproducibility 6
Sample Preparation 6
Tips for ELISA 7
ELISA FAQs 7
Contact us 9

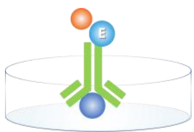
What's ELISA?



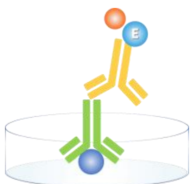
Enzyme-linked immunosorbent assay (ELISA) is an analytical biochemistry to detect ligand (mostly a protein) using antibodies directed against protein via a solid-phase type of enzyme immunoassay (EIA). In the most simple form of an ELISA, antigens from the sample (such as serum, plasma, cell culture supernatant, 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.

ELISA Types

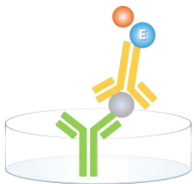
There are four main types of ELISA: direct ELISA, indirect ELISA, sandwich ELISA and competitive ELISA. Each has unique advantages and disadvantages.



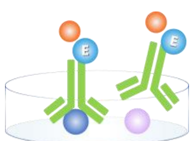
In a direct ELISA, an antigen or sample is immobilized directly on the plate and a conjugated detection antibody binds to the target protein. Substrate is then added, producing a signal that is proportional to the amount of analyte.
Suitable for: Analyzing the immune response to an antigen.



Indirect ELISA is a two-step ELISA which involves two binding process of primary antibody and labeled secondary antibody. The primary antibody is incubated with the antigen followed by the incubation with the secondary antibody.
Suitable for: The quantitative estimation of antibodies in the serum and other body fluids.



In a sandwich ELISA, the goal is to use antibodies to precisely quantify specific antigen present in a solution, such as a serum protein, or a hormone from the blood. The sandwich ELISA measures the amount of antigen between two layers of antibodies.
Suitable for: Determining analyte concentration in a biological sample.



Competitive ELISA is a technique used for the estimation of antibodies present in a specimen, such as serum. The sample antigen competes with a reference antigen for binding to a specific amount of labeled antigen.
Suitable for: When only one antigen is available for the antigen of interest. Also suitable for detecting small antigens that cannot be bound by the different antibodies.

Advantages and disadvantages

Type	Advantages	Disadvantages
Direct	• Only one antibody is used, so there is no cross-reaction problem and the reaction is fast.	• Antigen immobilization is not specific • when serum is used as the source of test antigen, all proteins in the sample may stick to the plate well, so small concentrations in serum must compete with other serum proteins binding to the well surface.
Indirect	• More than one labeled antibody is bound per antigen molecule. • Different primary detection antibodies can be used with a single labeled secondary antibody. • Fewer labeled antibodies are required.	• This may lead to nonspecific signals because of cross-reaction that the secondary antibody may cause.
Sandwich	• It has high specificity since two antibodies are used and antigen/analyte is specifically captured and detected. • It is suitable for complex samples as antigen does not require purification prior to measurement. • Has good flexibility and sensitivity, since both direct and indirect detection methods can be used.	• The antigen must have more than two antibody binding sites.
Competitive	• Less sensitive to sample dilution and sample matrix effects than the sandwich ELISA • Less variability between duplicate samples and assays • No sample processing is required and crude or impure samples can be used	• This test is done, generally, one test at a time and cannot be done with the microtiter plate. • The procedure is more complicated, and the product performance depends on the affinity of the antibody.

BT LAB ELISA portfolio

Sandwich ELISA

In a sandwich ELISA, the goal is to use antibodies to precisely quantify specific antigen present in a solution, such as a serum protein, or a hormone from the blood. The sandwich ELISA measures the amount of antigen between two layers of antibodies. The antigens to be measured must contain at least two antigenic sites, capable of binding to antibody, since at least two antibodies act in the sandwich. So, sandwich assays are restricted to the quantitation of multivalent antigens such as proteins or polysaccharides. Sandwich ELISAs for quantitation of antigens are especially valuable when the concentration of antigens is low and/or they are contained in high concentrations of contaminating protein.

Sandwich ELISA procedure:

1. Primary antibody (capture antibody) is added to the surface, and incubated to ensure primary antibody bound.
2. A blocking protein is added (e.g., albumin) to bind the remaining non-specific protein-binding sites in the well.
3. Then samples of unknowns are added to each antibody-coated well, and captured by antibody. Then the unbound antigen is removed by washing the wells.
4. Secondary antibody (detection antibody) is added, and binds to antigen (like a 'sandwich': the antigen is stuck between two antibodies). Then the unbound antibody-enzyme conjugates are removed by washing the wells.

5. A chemical is added to be converted by the enzyme into a color signal.
6. The absorbance of the plate wells is measured to determine the presence and quantity of antigen.

Featured product

Target	Product Code	Species
IL-6	E0004Ca, E0004Ch, E0028Go, E0090Hu, E0049Mo, E0122Po, E0003Rb, E0135Ra	H, M, R, Ca,G,R,P,C
TNF-A	E0019Ho, E0082Hu, E0117Mo, E0299Po, E0065Rb, E0764Ra, E0025Ca	H, M, R, Rb, F, Po, Hr
IL-1 beta	E0001Gp, E0143Hu, E0192Mo, E0119Ra	H, M, R, Gp
Leptin	E1559Hu, E0652Mo, E0561Ra, E0079Cat, E0026Ch, E0082Ca, E0062Sh	H, M, R, F, C, Ca, S
BDNF	E6633Hu, E0013Mo, E0476Ra, E0091Ho, E0228Rb, E2190Bo, E0537Po	H, M, B, R, Hr, Rb, Po
VEGF	E0080Hu, E0114Mo, E0659Ra, E2102Bo, E0019Ca, E0269Po	H, M, R, B, F, P
TGF-beta 1	E0134Hu, E0660Mo, E0304Po	H, M, P
INS	E0010Hu, E0283Po, E0707Ra, E0015Bo, E0074Gp,	H, B, G, P, R
CRP	E1798Hu, E0218Mo, , E0053Ra, E0150Gp, E0042Ha, E0048Po, E0016Rb	H, M, R, Gp, Hm, P, Rb
IFN-gamma	E0105Hu, E0056Mo, E0103Ra, E0005Bo, E0011Ca, E0013Cat, E0030Go, E0101Ho, E0100Po, E0049Sh	H, M, R, C, F, G, Gp, Hm, P, Rb
EPO	E1029Hu, E0003Mo, E0293Ra, E2308Bo, E0209Ca, E0116HO, E0231Rb, E0087Sh	H, M, R, B, C, Hr, Rb, S
MCP-1/CCL2	E4830Hu, E1707Mo, E0194Ra, E0170HO	H, M, R, Hr
IFN-beta	E0154Hu, E0195Mo, E0095Ra, E0043Cat, E0189Ch	H, M, R, F, Ch

Target	Product Code	Species
IL-4	E0092Hu, E0051Mo, E0133Ra, E0036Bo, E0003Ca, E0046GO, E0120Po, E0002Rb, E0079Sh	H, M, R, B, C, G, P, Rb, S
Resistin	E0338Hu, E0263Mo, E0087Bo	H, M, B
G-CSF	E0121Hu, E0641Mo, E2212Bo, E0344Ca, E0563Po	H, M, B, C, P
Angiopoietin 2	E1221Hu, E0336Mo, E0668Ra, E0044Bo, E0039Ca, E0272Po	H, M, R, B, C, P
VCAM-1	E0203Hu, E2318Ra, E0020Ca, E0270Po	H, R, C, P
IL-10	E0102Hu, E0022Mo, E0108Ra, E0006Ca, E0030Cat, E0128HO, E0110Po, EA0015Ch, E0004Rb	H, M, R, C, F, Hr, P, Ch, Rb
IL-17A	E0047Hu, E1143Mo, E0116Ra, E0069Bo	H, M, R, B
FGF-21	E1983Hu, E1575Mo	H, M
MMP-9	E0936Hu, E0277Mo, E0321Ra, E2279Bo, E0357Ca	H, M, R, B, C
IGFBP3	E0391Hu, E0023Mo, E0713Ra, E0017Bo, E0289Po	H, M, R, B, P
IGF-1	E0103Hu, E0037Mo, E0709Ra, E0016Bo, E0177Ca, E0012GO, E0014HO, E0284Po, E0144Rb, E0097Sh	H, M, R, B, C, G, Hr, P, Rb, S
IL-8	E0089Hu, E0052Cat, E0161HO, E0123Po, E0044Rb, E0073Sh	H, F, Hr, P, Rb, Sh
IL-2	E0094Hu, E0053Mo, E0123Ra, E0201Ca, E0051Cat, E0034GO, E0051HO, E0118Po, E0134Rb,	H, M, R, C, F, G, Hr, P, Rb
C-Reactive Protein	E0218Mo, E0053Ra, E0048Po, E0016Rb	M, R, P, Rb

Species Key: H Human, M mouse, R Rat, B Bovine, Ca Canine, C Chicken, F Feline, Fi Fish, G Goat, Gp Guinea pig, Hr Horse, Hm Hamster, P Porcine, Rb Rabbit, S Sheep

Competitive ELISA

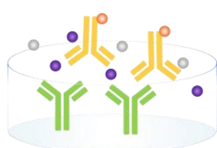
Competitive ELISA is a technique used for the estimation of antibodies present in a specimen, such as serum. The sample antigen competes with a reference antigen for binding to a specific amount of labeled antigen. The reference antibody is pre-coated on a multi-well plate and sample is pre-incubated with labeled antibody and added to the wells. The labeled antigen and the sample antigen (unlabeled) compete for binding to the

primary antibody. The lower the amount of antigen in the sample, the stronger the signal due to more labeled antigen in the well.

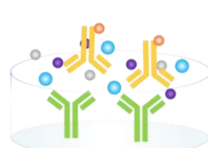
Competitive ELISA procedure:

1. Primary antibody is added to the sample which contains the antigen, and will bind the antigen forming an antibody-antigen complex.
2. The sample is then added to an antigen-coated well. Then the unbound antigen is removed by washing the wells. (The more antigen in the sample, the more Ag-Ab complexes are formed and so there are less unbound antibodies available to bind to the antigen in the well, hence "competition".)
3. Secondary antibody is added to the wells which binds the primary antibody. The secondary antibody is again bound to an enzyme which can catalyze a colorimetric or chemiluminescent reaction.

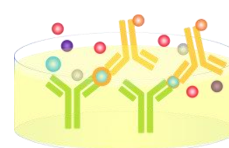
Antibody pre-coated on the plate



Sample and standard added



Biotinylated antigen added



Chemical added and color developing

Qualitative ELISA

As a method of direct ELISA, 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.

Competitve ELISA featured product

Target analyte	Product code	Species
CORT	EA0036Ra, EA0019Ch, EA0056Rb	R, C, Rb
GSH	EA0142Hu, EA0104Mo, EA0113Ra, EA0041Ca, EA0037Fi	H, M, R, F, Fi
PROG	EA0024Hu, EA0016Mo, EA0063Ra, EA0008Bo, EA0004Sh, EA0005Go	H, M, R, B, S, G
COR	EA0021Bo, EA0010Ge, EA0007Go	B, Ge, G
Estradiol	EA0030Hu, EA0011Ra, EA0018Sh, EA0016Fi	H, R, S, Fi
FSH	EA0014Mo, EA0015Ra	M, R
GH	EA0006Bo, EA0021Fi	B, Fi
Immunoglobulin G	EA0027Mo, EA0033Ra	M, R

Qualitative ELISA featured product

Target analyte	Product code	Species
Brucella Ab (IgG)	ED0470Hu, ED0080Mo, ED0023Bo, ED0004Ca, ED0000Go, ED0000Sh, ED0003Po	H, M, B, C, G, S, P
Helicobacter pylori (IgM)	ED0022Hu, ED0049Mo	H, M
Helicobacter pylori (IgM)	ED0023Hu	H
Hepatitis E (IgG)	ED0034Hu, ED0086Mo	H, M
Rotavirus antigen	ED0076Hu, ED0039Mo, ED0018Bo, ED0001Ca, ED0003Go, ED0017Rb,	H, M, B, C, G, Rb
0Papiloma virus Ab (IgG)	ED0022Ca	C
Aquaporin 4(AQP4) Ab	ED0546Hu	H
Tuberculosis Ab (IgG)	ED0324Hu	H

Species Key: H Human, M mouse, R Rat, B Bovine, C Chicken, F Feline, Fi Fish, G Goat, Ge General, Gp Guinea pig, P Porcine, Rb Rabbit, S Sheep

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 detects the target protein specifically without cross-reacting with non-specific 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.

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.

Sample Preparation

Serum Allow serum to clot for 10-20 minutes at room temperature. Centrifuge at 1,000 x g 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 1,000 x g. 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 minutes. 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 1,000 x g 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 1,000 x g 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 1,000 x g for 20 minutes to remove particulate matter. Assay immediately or aliquot and store at ≤ -20 °C.

Ascites/Cerebrospinal fluid Collect by sterile tube. Centrifuge at 1,000 x g 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 avoid cross-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.
- When pipetting, dispense liquid with the pipette tips held at an angle and not touching the bottom of the well.
- 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 supernatants. 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 40ul.

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 BT Lab'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.

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