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Elabscience®

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ELISA Guide

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ABOUT US

Elabscience® empowers researchers in Cell Detection Areas by providing high-quality reagents and services. Researchers in more than 150 countries and regions use our high quality and innovative tools to explore the mysteries of cellular functions. Our product expertise covers a diverse set of Flow Cytometry Antibodies, Cell Function Assays, Cell Metabolism Assays, ELISA Kits, Proteins and Antibodies. All of these products have passed ISO9001 international certification and EU CE certification.

45
Authorized Patents

15,000+
SCI Publications

76,000+
Total IF

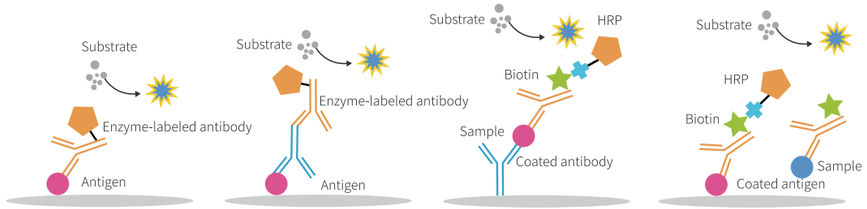
Content

Enzyme Linked Immunosorbent Assay (ELISA)

Enzyme Linked Immunosorbent Assay (ELISA) is the most widely used immunological assay technique. It combines the specificity of antigen-antibody reaction with the efficiency of enzyme catalysis, and determines the result by the chromogenic reaction after enzyme action on the substrate. The absorbance (OD value) is generally measured with an enzyme-labeled instrument to reflect the concentration of antigen or antibody. The sensitivity can reach the level of nanogram (ng) per milliliter or even picogram (pg). Due to the high catalytic efficiency of the enzyme, which indirectly amplifies the results of the immune response, the assay can achieve high sensitivity.

I ELISA Experimental Principles

At present, the commonly used ELISA methods include direct, indirect, sandwich and competitive. The sandwich ELISA is generally used for the detection of large molecular proteins, while competitive ELISA is generally used for the detection of small molecular proteins or compounds.



Direct ELISA | Indirect ELISA | Sandwich ELISA | Competitive ELISA

Direct ELISA

Principle

A method of detecting antigen content using the specific reaction between solid phase antigen and enzyme-labeled primary antibody. Because the enzyme-conjugate antibody reacts directly with the solid phase antigen, it is called the direct method.

Indirect ELISA

Principle

Indirect ELISA is a common method to detect antibodies. The principle of this method is to use the enzyme labeled secondary antibody to detect the antibody that binds to the solid phase antigen, so it is called indirect method.

Sandwich ELISA

Principle

Sandwich method is divided into double antibody sandwich and double antigen sandwich, which is suitable for the detection of large molecular proteins with multiple recognition sites.

The principle of double-antibody sandwich ELISA is to bind the specific antibody to the solid phase carrier to form the solid phase antibody, combine with the corresponding antigen in the sample to be tested to form the antigen-antibody immune complex, then add the enzyme-labeled antibody, combine with the antigen in the immune complex to form the solid phase antigen-antigen-enzyme labeled antibody complex, add the substrate to show color, and finally determine the antigen content. The final chromogenic result is proportional to the amount of antigen to be detected.

The response pattern of double-antigen sandwich ELISA is similar to that of double-antibody sandwich ELISA. The antibody content in the sample can be determined by using solid phase antigen and enzyme-labeled antigen to replace solid phase antibody and enzyme-labeled antibody respectively. The final chromogenic result is proportional to the amount of antibody to be detected.

Competitive ELISA

Principle

Competitive is mainly used to detect small molecular proteins with only one recognition site. The principle is to bind the specific antigen to the solid phase carrier to form the solid phase antigen. At the same time, the sample to be tested and the enzyme-labeled antibody are added. The antigen and the solid phase antigen in the sample to be tested will competitively bind to the enzyme-labeled antibody, and then the substrate is added for color development. The more antigens in the sample to be tested, the less solid antigen bound to the enzyme-labeled antibody, and the lighter the color. The final color result is inversely proportional to the amount of antigen (or antibody) to be tested.

Comparison of Four ELISA Methods

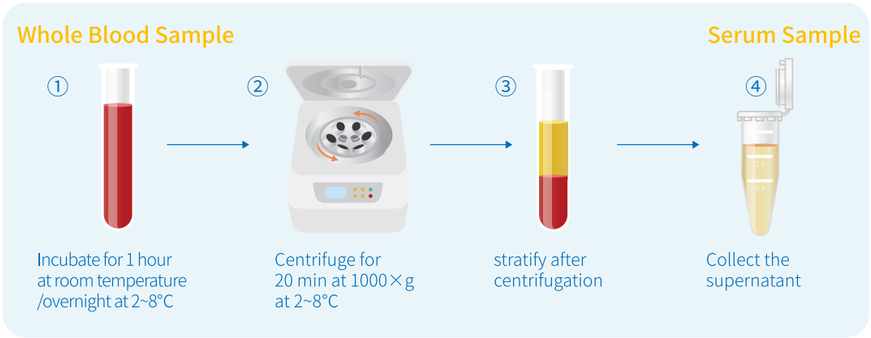
Classification of Methods	Direct ELISA	Indirect ELISA	Sandwich ELISA	Competitive ELISA
Applicability	It is rarely used in practice and can only measure enzyme-labeled molecules.	It is an important means to detect marker antibodies in clinical diagnosis.	It is suitable for the detection of large molecular proteins with multiple recognition sites.	It is suitable for the determination of small molecular antigens, such as hormones and drugs.
Advantages	① Fewer experimental steps and faster detection speed; ② It does not need to use the secondary antibody,avoids the cross reaction, and the determination result is not prone to error.	① The primary antibody without enzyme labeling can retain more immunoreactivity and has higher sensitivity; ② Less labeled antibodies are required (the secondary antibody is generally a multi-antibody, which can mark multiple enzyme molecules) and the Indirect ELISA is more economical.	High sensitivity, high specificity, antigen doesnot need prior purification.	It can be applied to relatively impure samples with high data reproducibility.
Disadvantages	① Due to the non-specific fixation of antigens, the experimental background will be relatively high and the sensitivity will be low; ② The detection of target molecules is limited, and the Direct ELISA only can determine the enzyme-labeled molecules.	① The probability of cross reaction is higher (the enzyme-labeled secondary antibody binds to the antigen directly); ② The experiment period was prolonged due to the addition of secondary antibody incubation steps.	① The antigen must have more than two antibody binding sites; ② High requirements for paired antibodies should be optimized to reduce the cross-reactivity between capture antibodies and detection antibodies in advance.	The overall sensitivity and specificity is poor.

II ELISA Common Sample Processing Methods

There are many types of samples used for ELISA experiments, including serum, plasma, cell culture supernatants, tissue homogenates, saliva and urine. The pretreatment methods of different sample types are different. Proper sample pretreatment is the first step to ensure the accuracy of ELISA experiments.

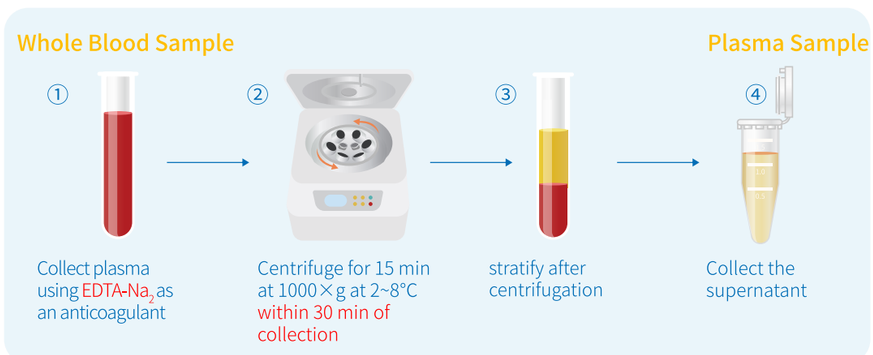
Serum

Collect the blood samples using blood collection tubes without anticoagulants. Allow samples to clot for 1 hour at room temperature or overnight at 4℃ before centrifugation for 20 min at 1000×g at 2~8℃. Collect the supernatant to carry out the assay.



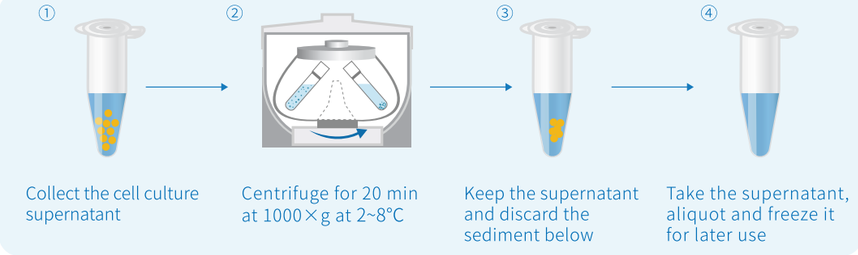
Plasma

Collect the blood samples using blood collection tubes containing anticoagulants, and EDTA-Na_2 is recommended as anticoagulant. Centrifuge samples for 15 min at 1000×g at 2~8℃ within 30 min of collection. Collect the supernatant to carry out the assay.



Cell Culture Supernatants

The cell culture supernatants were sucked into the centrifuge tube. Centrifuge samples for 15 min at 1000 × g at 2~8°C, remove cell debris and impurities. Collect the supernatant to carry out the assay.

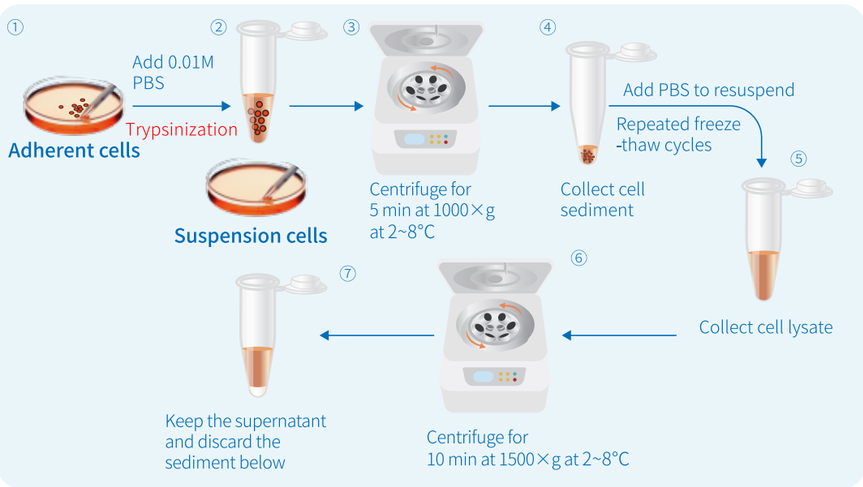


Cell Lysates

①The adherent cells were gently cleaned with cold PBS, digested with trypsin, centrifuged for 5 min at 1000×g and then collected; Suspension cells can be collected directly by centrifugation;

②The collected cells were washed 3 times with cold PBS. In each 10^6 cells, 150~200 μL PBS was added for re-suspension (it is recommended to add protease inhibitors to PBS, if the content is very low, the volume of PBS can be reduced), and the cells were broken by repeated freeze-thaw or ultrasound;

③The extraction solution was centrifuged for 10 min at 1500×g at 2~8°C, and the supernatant was detected.



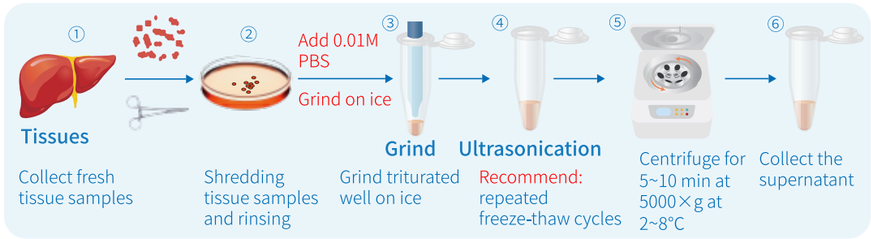
Tissue Homogenates

①Rinse the tissue with pre-cooled PBS (0.01 M, PH 7.4) to remove the residual blood or impurities on the surface;

②Weigh the tissue block and cut it into pieces as small as possible so that it can be fully homogenized;

③Add an appropriate amount of pre-cooled PBS (generally according to the weight to volume ratio of 1:9, such as 1 g tissue sample corresponding to 9 mL PBS, the specific volume can be adjusted according to the needs of the experiment, and make a record. It is recommended to add protease inhibitors to PBS) and fully homogenize in ice or ice bath with a glass homogenizer. In order to further lysate the tissue cells, the homogenate can be broken by ultrasound or frozen and thawed repeatedly;

④The homogenate was sucked into the centrifuge tube, centrifuged for 5~10 min at 5000×g at 2~8°C. Collect the supernatant to carry out the assay.



Urine

Urine samples were collected in a sterile container and centrifuged for 20 min at 1000×g at 2~8°C to remove impurities. Collect the supernatant to carry out the assay.

Saliva

Saliva was collected using a sterile EP tube and centrifuged for 10 min at 4000×g at 2~8°C to remove impurities. Collect the supernatant to carry out the assay. Fresh saliva samples should be used.

Milk

The milk sample was collected in a sterile container and centrifuged for 15 min at 1000×g at 2~8°C, the clarified part was taken, and the process was repeated twice. Collect the clarified liquid to carry out the assay.

Feces

Feces samples were collected in a sterile container and PBS buffer was added to the feces at a ratio of 9 mL/g (0.01 M, pH 7.4; optionally add 0.05 M EDTA) and oscillate on ice for 15 min. Centrifuge it for 5~10 min at 5000×g at 2~8°C, and then collect the supernatant to carry out the assay.

Other Types of Biological Fluids

After liquid collection, centrifuge for 20 min at 1000×g at 2~8°C to remove impurities and cell fragments, and then collect the supernatant to carry out the assay.

Sample Precautions

Sample Collection

When collecting blood samples, hemolysis should be avoided as much as possible, and bacterial contamination should also be avoided to avoid false positive results.

Avoid using hyperlipemic samples. Lipids affect the homogeneity of samples and the binding of antigens and antibodies, resulting in a decrease in the accuracy of measurements.

Sample Storage

After sample collection and treatment, if the sample is assayed within 1 week, it can be stored in 2~8°C; and if it cannot be assayed in time, please aliquot it according to the amount of one time and freeze at -20°C (test within 1 month) or -80°C (test within 3 months). Avoid repeated freeze-thaw cycles. Centrifuge again before assaying to remove any additional precipitates that may appear after storage.

Sample Dilution

The detection range of the kit is different from the concentration range of the sample. If the concentration of the substance to be measured in the sample is higher than the highest value of the standard, please make an appropriate dilution according to the actual situation (Suggest consulting literature and doing pre-experiments).

Sample Composition

Commercial lysis reagents are not recommended when handling cell extracts or tissue samples. Some components of lysis reagents may disrupt the spatial conformation of proteins, affect the binding of antigens and antibodies, or cause matrix interference, and ultimately affect the experimental results.

Sample Dilution Method

Please estimate the concentration range of the sample in advance, and determine the dilution ratio of the sample to be measured by pre-experiment or consulting technical support.

If your test sample needs dilution, refer to the dilution method as follows:

① **For 100 fold dilution**: One-step dilution. Add 5 µL sample to 495 µL sample diluent to yield 100 fold dilution.

② **For 1000 fold dilution**: Two-step dilution. Add 5 µL sample to 95 µL sample diluent to yield 20 fold dilution, then add 5 µL 20 fold diluted sample to 245 µL sample diluent, after this, the neat sample has been diluted at 1000 fold successfully.

③ **For 100000 fold dilution**: Three-step dilution. Add 5 µL sample to 195 µL sample diluent to yield 40 fold dilution, then add 5 µL 40 fold diluted sample to 245 µL sample diluent to yield 50 fold dilution, and finally add 5 µL 2000 fold diluted sample to 245 µL sample diluent, after this, the neat sample has been diluted at 100000 fold successfully.

The amount of liquid taken at each dilution step is not less than 3 µL, and the dilution ratio is not more than 100 times. Each dilution should be mixed evenly to avoid bubbles.

Assay Procedure for ELISA

Direct ELISA

Direct method is rarely used in practice and can only measure enzyme-labeled molecules. The ELISA kits developed by Elabscience® do not contain direct method principle kits (the following direct method procedure is for reference only).

Before the experiment, the reagents should be balanced to room temperature (reagents cannot be dissolved directly at 37°C). When diluting reagents or samples, ensure that they are well mixed and avoid bubbles as much as possible.

Assay Procedure

① **Add Sample**: Each well (except blank) was added with HRP enzyme-labeled antibody 100 µL. Cover the plate with the sealer provided in the kit, and incubate for 60 min at 37°C.

② **Wash Plate**: Decant the solution from each well, add 350 µL of wash buffer to each well. Soak for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 5 times.

(Note: After washing the plate, please proceed to the next step immediately, do not let the ELISA plate dry.)

③ **Add Substrate**: Add 90 µL of Substrate Reagent (TMB) to each well (including blank). Cover the plate with the sealer provided in the kit, and incubate for about 15 min at 37°C. Protect the plate from light.

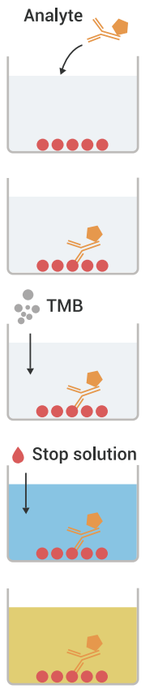
(Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement.)

④ **Add Stop Solution**: Add 50 µL of Stop Solution to each well (including blank) in order to stop the reaction.

(Note: The order to add stop solution should be the same as the substrate solution.)

⑤ **OD Measurement**: Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Assay Procedure Summary for Direct ELISA



1. Add 100 µL HRP-conjugate working solution to the wells. Incubate for 60 min at 37°C.

2. Aspirate and wash the plate for 5 times.

3. Add 90 µL Substrate Reagent. Incubate for 15 min at 37°C.

4. Add 50 µL Stop Solution.

5. Read the plate at 450 nm immediately. Calculation of the results.

Indirect ELISA

Assay Procedure

① **Add Sample**: Determine wells for positive control, negative control, blank and sample. Add 100 µL control product and 100 µL sample to be tested into the appropriate wells, and add no additional reagents except substrate reagent and stop in the blank wells. Cover the plate with the sealer and incubate at 37°C for 45 min.

(Note: Solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible. The sample addition time should be controlled within 10 min.)

② **Wash Plate**:Decant the solution from each well, add 350 µL of wash buffer to each well. Soak for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times.

(Note: After washing the plate, please proceed to the next step immediately, do not let the ELISA plate dry.)

③ **Add Enzyme-Labeled Antibody**: Add 100 µL of HRP Conjugate working solution to each well (except blank). Cover the plate with anew sealer. Incubate for 30 min at 37°C.

④ **Wash Plate**: Decant the solution from each well, repeat the wash process for 5 times as conducted in step 2.

⑤ **Add Substrate**: Add 50 µL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37°C. Protect the plate from light.

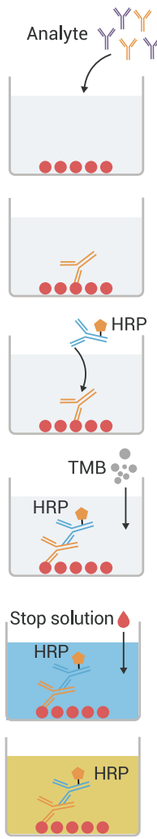
(Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement.)

⑥ **Add Stop Solution**: Add 50 µL of Stop Solution to each well (including blank) in order to stop the reaction.

(Note: The order to add stop solution should be the same as the substrate solution.)

⑦ **OD Measurement**: Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Assay Procedure Summary for Indirect ELISA



1. Add 100 µL of controls and samples to the appropriate wells. Incubate for 45 min at 37°C.

2. Aspirate and wash the plate for 3 times.

3. Add 100 µL of HRP Conjugate working solution.Incubate for 30 min at 37°C. Aspirate and wash the plate for 5 times.

4. Add 90 µL of Substrate Reagent. Incubate for about 15 min at 37°C.

5. Add 50 µL of Stop Solution.

6. Read the plate at 450 nm immediately. Calculation of the results.

Sandwich ELISA

Before the experiment, the reagents should be balanced to room temperature (reagents cannot be dissolved directly at 37°C). When diluting reagents or samples, ensure that they are well mixed and avoid bubbles as much as possible.

Assay Procedure

① **Add Sample**: Determine wells for diluted standard, blank and sample. Add 100 µL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate). Cover the plate with the sealer provided in the kit. Incubate for 90 min at 37°C.

(Note: Solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible. The sample addition time should be controlled within 10 min.)

② **Add Detection Antibody**: Decant the liquid from each well, do not wash. Immediately add 50 µL of Biotinylated Detection Ab working solution to each well. Cover the plate with a new sealer. Incubate for 1 hour at 37°C.

③ **Wash Plate**: Decant the solution from each well，add 350 µL of wash buffer to each well. Soak for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times.

(Note: After washing the plate, please proceed to the next step immediately, do not let the ELISA plate dry.)

④ **Add HRP Conjugate**: Add 50 µL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 min at 37°C.

⑤ **Wash Plate**: Decant the solution from each well, repeat the wash process for 5 times as conducted in step 3.

⑥ **Add Substrate**: Add 50 µL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37°C. Protect the plate from light.

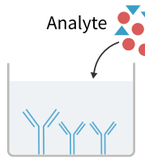
(Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement.)

⑦ **Add Stop Solution**: Add 50 µL of Stop Solution to each well in order to stop the reaction.

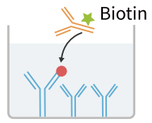
(Note: The order to add stop solution should be the same as the substrate solution.)

⑧ **OD Measurement**: Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

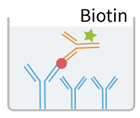
Assay Procedure Summary for Sandwich ELISA



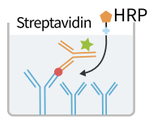
1. Add 100 µL standard or sample to the wells. Incubate for 90 min at 37°C.



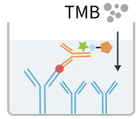
2. Discard the liquid, immediately add 100 µL Biotinylated Detection Ab working solution to each well. Incubate for 60 min at 37°C.



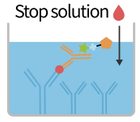
3. Aspirate and wash the plate for 3 times.



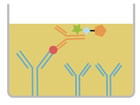
4. Add 100 µL HRP conjugate working solution. Incubate for 30 min at 37°C. Aspirate and wash the plate for 5 times.



5. Add 90 µL Substrate Reagent. Incubate for 15 min at 37°C.



6. Add 50 µL Stop Solution.



7. Read the plate at 450 nm immediately. Calculation of the results.

Competitive ELISA

Before the experiment, the reagents should be balanced to room temperature (reagents cannot be dissolved directly at 37°C). When diluting reagents or samples, ensure that they are well mixed and avoid bubbles as much as possible.

Assay Procedure

① **Add Sample and Biotinylated Detection Ab**: Determine wells for diluted standard, blank and sample. Add 50 µL each dilution of standard, blank and sample into the appropriate wells (It is recommended that all samples and standards be assayed in duplicate). Immediately add 50 µL of Biotinylated Detection Ab working solution to each well. Cover the plate with the sealer provided in the kit. Incubate for 45 min at 37°C.

(Note: Solutions should be added to the bottom of the micro ELISA plate well, avoid touching the inside wall and causing foaming as much as possible. The sample addition time should be controlled within 10 min.)

② **Wash Plate**: Decant the solution from each well，add 350 µL of wash buffer to each well. Soak for 1 min and aspirate or decant the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 3 times.

(Note: After washing the plate, please proceed to the next step immediately, do not let the ELISA plate dry.)

③ **Add HRP Conjugate**: Add 100 µL of HRP Conjugate working solution to each well. Cover the plate with a new sealer. Incubate for 30 min at 37°C.

④ **Wash Plate**: Decant the solution from each well, repeat the wash process for 5 times as conducted in step 2.

⑤ **Add Substrate**: Add 90 µL of Substrate Reagent to each well. Cover the plate with a new sealer. Incubate for about 15 min at 37°C. Protect the plate from light.

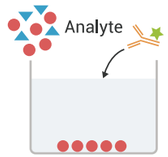
(Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 min. Preheat the Microplate Reader for about 15 min before OD measurement.)

⑥ **Add Stop Solution**: Add 50 µL of Stop Solution to each well in order to stop the reaction.

(Note: The order to add stop solution should be the same as the substrate solution.)

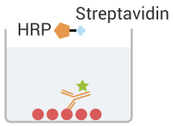
⑦ **OD Measurement**: Determine the optical density (OD value) of each well at once with a micro-plate reader set to 450 nm.

Assay Procedure Summary for Competitive ELISA

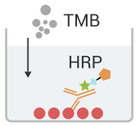


1. Add 50 µL standard or sample to the wells, immediately add 50 µL Biotinylated Detection Ab working solution to each well. Incubate for 45 min at 37°C.

2. Aspirate and wash the plate for 3 times.



3. Add 100 µL HRP conjugate working solution. Incubate for 30 min at 37°C. Aspirate and wash the plate for 5 times.



4. Add 90 µL Substrate Reagent. Incubate for 15 min at 37°C.

5. Add 50 µL Stop Solution.

6. Read the plate at 450 nm immediately. Calculation of the results.

Precautions for ELISA Experimental Operation

Preparation for kit

The most important assay tool in the experiment is the ELISA kit. For the ELISA kit,carefully read the manual,focus on the composition and storage conditions of the kit, precautions and product performance, check whether the kit validity period is within the shelf life, whether the reagent solution of each component is clarified enough, and whether it can be mixed.

If the above abnormal conditions occur, a new ELISA kit should be replaced.

Note: Before the experiment, all reagent components of kit should be balanced to room temperature (18~25℃). The working and washing solution in the process should be used on the spot.

Adding sample

The key of repeated accuracy in pipetting is consistent technique. Consistent speed and smoothness is necessary during pipetting.

When adding samples, the sample should be added at the bottom of the ELISA plate well, avoiding the upper part of the well wall. In particular, it is necessary to pay attention to the fact that it cannot splash out or produce bubbles.

It is generally recommended that standards and samples be added with a micro sampler. The pipette tip should be replaced each time the sample is added to avoid cross-contamination. When adding standard, it is necessary to follow the principle of "adding the concentration from low to high, and adding the same concentration in parallel".

When adding HRP Conjugate working solution, substrate and stop solution, a quantitative multi-channel pipettes can be used to complete the liquid adding process quickly. It is recommended that the sampling time of the full plate should not exceed 10 min.

Incubation

Before the incubation operation, it is necessary to check if there are any omissions in the sample addition. If not, it is necessary to paste a suitable size of sealing film on the ELISA plate and mark it accordingly. Hold the ELISA plate horizontally on the both sides of the frame and place it in a 37°C incubator for incubation.

During the incubation process, the incubation temperature and incubation time must be strictly controlled. Each incubation requires a new sealing film.

Washing

The specific operation of washing includes discarding liquid and washing, and attention should be paid to:

Discarding Operation: After removing ELISA plate from the incubator, **the liquid in the well of ELISA plate should be quickly and vertically drained to avoid cross-contamination between the wells, and then pat it dry on a clean absorbent paper.** It' s recommended to replace the absorbent paper each time, avoid repeated clappers at the same position, and do not allow the liquid in the well dry naturally.

Washing Operation: It can be washed manually or by washing machine. Wash the plate 3 or 5 times according to the instructions. During the washing process, the prepare the washing liquid should be used on the spot when needed to avoid pollution. At the same time, attention should be paid to controlling the volume of washing liquid, washing times and soaking time.

Coloration and Colorimetry

Coloration is the final incubation reaction in the ELISA experiment. Before adding the sample, observe the color of the chromogenic solution. If it shows light blue or blue, it needs to be replaced with a new colorless transparent chromogenic solution immediately. The chromogenic reaction should be carried out away from light, and the reaction temperature and reaction time should be controlled according to the protocol.

Colorimetry is the final step in the entire ELISA experiment. The OD values of each well should be measured at 450 nm using microplate reader immediately after the reaction is stopped by adding the stop solution.

Before assay, it should be noted that the microplate reader should be opened 15 min in advance to preheat, set the corresponding parameters, and ensure that the bottom of the ELISA plate is dry and clean. If there are water stains, gently wipe to keep clean.

Ⅳ Calculation of ELISA Results

In the ELISA experiment, the standard or sample is usually set up 1 or 2 multiple wells for assay, and the average OD value of the standard and sample is calculated separately.

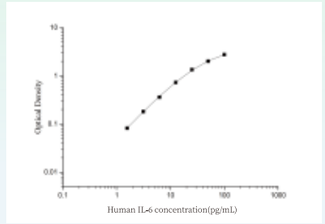
① **Sandwich ELISA Kit**: The OD average of standard and sample should be subtracted from the OD average when the concentration of standard is zero to obtain the correction value.

② **Competitive ELISA Kit**: The OD average of the standard and sample does not need to subtract the OD average when the standard concentration is zero.

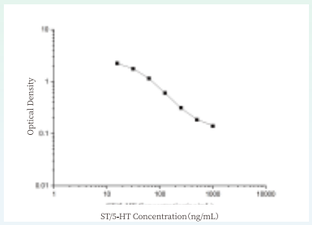
③ **Standard Curve Fitting**: Plot a four parameter logistic curve on log-log axis using the mapping software, with standard concentration on the X-axis and OD values on the Y-axis, and this curve is the standard curve.

④ **Sample Concentration Calculation**: Bring the OD value of sample into the calibration curve to obtain the corresponding concentration. If the sample has been diluted before the experiment, the actual concentration of the sample should be the concentration obtained on the standard curve multiplied by the dilution ratio.

ELISA Standard Curve

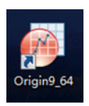


Sandwich Method



Competitive Method

The Origin software is recommended for fitting.



For details about Origin, please refer to the official website:
www.elabscience.com/resource-elisa_research-detail-1132

Ⅴ Analysis of ELISA Results

1.Interpretation of Initial Data

① OD value of Blank Control ⩽ 0.1 (Sandwich Method), the maximum OD value of Standard Curve is generally above 1.2.

② The sample OD value should fall within the range of the standard curve concentration.

③ Precision (SD/Mean*100%) ⩽10%.

④ Linearity ⩾ 0.99.

Sample layout	1	2	3
A	standard 1	standard 1	Serum(neat)
B	standard 2	standard 2	Serum(neat)
C	standard 3	standard 3	Serum(1:50)
D	standard 4	standard 4	Serum(1:50)
E	standard 5	standard 5	Serum(1:1000)
F	standard 6	standard 6	Serum(1:1000)
G	standard 7	standard 7	--
H	blank 1	blank 2	--

OD value	1	2	3
A	2.289	2.263	2.972
B	1.627	1.617	3.013
C	1.104	1.088	0.886
D	0.651	0.612	0.893
E	0.382	0.375	0.059
F	0.211	0.202	0.067
G	0.119	0.115	--
H	0.040	0.042	--

Note:

1) OD value of standard curve: 0~3.5.

2) The calibration OD value should be used to fit the standard curve of the sandwich method and calculate the sample concentration.

3) It is not recommended to use Excel table to fit standard curve.

4) If the dilution of the sample is too large, it is recommended to use stepwise dilution.

2. Common Abnormal Results

① Overall OD Value is Low or No Signal

Possible Causes	Solutions
a. The kit has lost its activity.	Use a new kit and store the kit according to the instructions.
b. Kit components are not balanced to room temperature.	Each component of the kit needs to be balanced to room temperature for testing.
c. Mixed with other reagents or didn't mix up well.	Please do not mix use the reagents from other brand or different batch to avoid mismatch issue. Ensure the solution is well mixed.
d. Wash buffer was contaminated or wash the plate improperly.	Prepare fresh wash buffer, operate according to manual.
e. Insufficient incubation time.	Note that the three incubation times in the experiment are 90 min, 60 min and 15 min respectively.
f. Incubation temperature is unstable.	Check the incubator to ensure that the incubation temperature is stable at 37°C .
g. Biotinylated Detection Ab or HRP enzyme conjugate is insufficient.	Ensure accurate concentration extraction amount, completely dilute and prepare the working fluid 15 min in advance.
h. Biotinylated Detection Ab or HRP conjugates are diluted incorrectly or the steps are reversed.	① Firstly, the performance of HRP concentrate was judged by whether the color changed after mixing the substrate with HRP concentrate. ② Then experiment was repeated according to the correct steps when the performance of HRP concentrate was normal.
i. Stop solution is not added.	Stop solution should be added to each well before measurement.

② OD Value of Standard Curve is Abnormal

1) Standard Curve dilution linearity is poor.

Standard	
2.590	2.598
1.560	1.639
1.222	1.240
0.611	0.581
0.661	0.618
0.207	0.183
0.150	0.155
0.089	0.086

Possible Causes	Solutions
a. Standard is insufficiently mixed.	Ensure thoroughly mixing in each dilution step (about 20 times).
b. Pipette volumes are not accurate.	① Using calibrated pipettes. ② Adding samples vertically and quickly. ③ Do not touch the pipette tips to the wall or the bottom of wells.
c. Cross contamination between wells.	Act quickly when discarding liquid, tap dry the plate, replace the absorbent paper.
d. Insufficient washing.	① Ensure sufficient washing. ② If using an automatic plate washer, check that all ports are clean and unobstructed.
e. Incubation temperature is incorrect.	① Adhere to recommended incubation temperature. ② Avoid putting plates in variable environmental conditions.
f. There are bubbles in wells.	① When adding liquid, it should be regulated to avoid bubbles. ② The working solution can be centrifuged to reduce the formation of bubbles.

2) Standard Curve shows low signal.

Standard	
0.585	0.706
0.341	0.307
0.211	0.226
0.112	0.122
0.082	0.091
0.075	0.066
0.07	0.062
0.053	0.073

Possible Causes	Solutions
a. Improper storage of the kit.	Store the kit strictly according to the instructions.
b. The reagent is not balanced to room temperature before use.	Before the experiment, place the kit at room temperature for about 20 min.
c. The working solution takes a long time to configure.	The working solution should be configured about 15 min before use.
d. The dilution ratio of the working solution is incorrect.	It is necessary to configure 100× working solution into 1×.
e. Insufficient reagent volume or missed addition.	Make sure all reagents are added in order and in sufficient quantities.
f. Incubation time/temperature did not meet the requirements.	Check the incubator to ensure that the incubation temperature is stable at 37°C .
g. The substrate coloration time is short and the color development is insufficient.	The color development time can be extended, generally not more than 25 min.

③ OD Value of the Sample is Abnormal

1) OD value of Standard Curve is normal, while the sample shows low signal.

Standard		Sample	
2.442	2.439	0.058	0.05
1.801	1.799	0.053	0.041
1.165	1.164	0.046	0.063
0.639	0.641	0.039	0.057
0.411	0.409	0.044	0.034
0.347	0.341	0.048	0.059
0.258	0.275	0.052	0.054
0.046	0.033	0.035	0.045

Possible Causes	Solutions
a. Matrix effect.	Avoid introducing other-sourced substrates.
b. Hook effect.	① Dilute the sample. ② Search the literature, and determine the optimal dilution ratio of the sample through pre-experiment.
c. Insufficient abundance of target protein.	Concentrate the sample or choose a more sensitive kit.
d. Improper handling or storage of samples.	① Ensure samples are collected and stored correctly to avoid repeated freezing and thawing. ② Remelted samples need to be centrifuged to obtain supernatant and balanced to room temperature before testing.
e. Sample type not applicable.	① An ELISA kit that matches the type of test sample should be selected. ② Avoid introducing other-sourced substrates.

2) OD value of standard curve is normal, while the sample shows high signal.

Standard		Sample	
2.442	2.439	3.165	3.723
1.801	1.799	2.341	2.307
1.165	1.164	3.411	3.426
0.639	0.641	3.412	3.422
0.411	0.409	2.827	2.791
0.347	0.341	1.375	1.366
0.258	0.275	1.607	1.662
0.046	0.033	1.253	1.173

Possible Causes	Solutions
a. Sample concentration is too high.	① Dilute the sample. ② Search the literature, and determine the optimal dilution ratio of the sample through pre-experiment.
b. Improper handling or storage of samples (Contains endogenous components that interfere with color development).	① Ensure samples are collected and stored correctly to avoid hemolysis, contamination and multiple freeze-thaw cycles. ② The influence of slight hemolysis on test results can be reduced by sufficient washing operation.
c. Matrix effect.	① Avoid introducing other-sourced substrates. ② Diluting the sample can reduce the matrix effect. ③ Sufficient washing can reduce the influence of endogenous substrates.

④ Standard/Sample Duplicate Wells Vary Greatly

Standard		Sample	
2.458	2.306	0.765	0.423
1.566	1.327	0.341	0.307
0.953	0.945	0.211	0.426
0.46	0.852	0.412	0.422
0.277	0.264	0.827	0.791
0.168	0.174	0.675	0.366
0.12	0.137	0.607	0.662
0.046	0.053	0.453	0.473

Precision CV≤10%

Possible Causes	Solutions
a. Unequal volumes added to wells.	① Using calibrated pipettes. ② Adding samples vertically and quickly.
b. When adding sample, it is added to the non-coated area on the upper part of the well wall.	Do not touch the pipette to the wall or the bottom of wells.
c. Mixed with other brands of kit reagents.	Please do not mix use the reagents from other brand or different batch to avoid mismatch issue.
d. Samples/reagents are insufficiently mixed.	Make sure the sample and reagent are fully mixed.
e. Incomplete washing plate.	Ensure washing process and time according to the instructions.
f. The sequence of adding the substrate solution and the stop solution was not consistent.	Ensure consistency in the sequence of all filling steps.
g. There are bubbles in the wells.	① When adding liquid, it should be regulated to avoid bubbles. ② The working fluid can be centrifuged to reduce the formation of bubbles.
h. Kit components are not balanced to room temperature.	Each component of the kit needs to be balanced to room temperature for testing.
i. Improper storage of samples.	Ensure samples are collected and stored correctly to avoid repeated freezing and thawing.
j. Insufficient stop steps.	The terminated solution needs to be read after completing the vibration plate operation.

⑤ The Blank Well Background Value is High

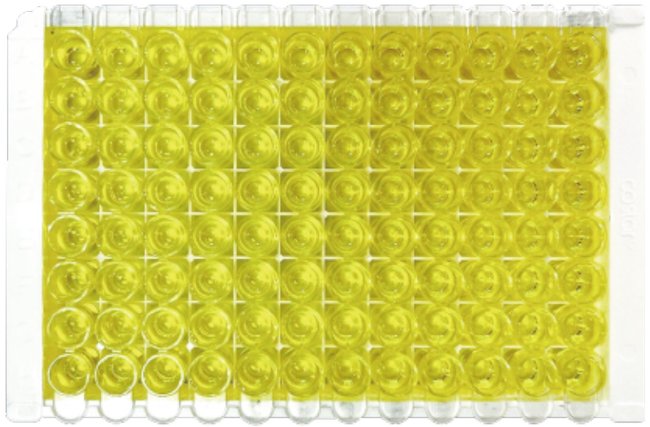
Standard		Sample	
2.442	2.439	0.765	0.723
1.801	1.799	0.341	0.307
1.165	1.164	0.411	0.426
0.639	0.641	0.412	0.422
0.411	0.409	0.827	0.791
0.347	0.341	0.375	0.366
0.258	0.275	0.607	0.662
0.184	0.202	0.253	0.173

Blank Control

OD value of blank control needs ⩽0.1 (Sandwhich Method)

Possible Causes	Solutions
a. The kit has lost its activity.	Use a new kit and store the kit according to the instructions.
b. Kit components are not balanced to room temperature.	Each component of the kit needs to be balanced to room temperature for testing.
c. Mixed with other brands of kit reagents.	Please do not mix use the reagents from other brand or different batch to avoid mismatch issue.
d. Contaminated wash buffer.	Prepare fresh wash buffer.
e. Detection antibody/enzyme conjugate working solution concentration is too high.	Use the dilution ratio recommended in the instruction.
f. Inadequate washing of the ELISA plate.	Ensure that each step is washed completely, and use the washing solution equipped with the kit when washing the plate to prevent cross contamination.
g. Incomplete tapping.	Fully tap until there is no obvious liquid remaining in the well.
h. Incubation temperature is too high.	Check the incubator to ensure that the incubation temperature is stable at 37°C .
i. Incubation time is too long.	Incubate according to the reaction time specified in the instructions.

⑥ Overall Standard Curve and Sample is Positive



Possible Causes	Solutions
a. The kit components are contaminated.	Use a new kit and store the kit according to the instructions.
b. Reagent preparation is incorrect.	Prepare the corresponding reagent solution according to the steps in the kit instructions.
c. Pipette volumes are not accurate.	① Using calibrated pipettes. ② Add samples vertically and quickly. ③ Do not touch the pipette to the wall or the bottom of wells.
d. Mixed with other brands of kit reagents.	Please do not mix use the reagents from other brand or different batch to avoid mismatch issue.
e. Insufficient washing times.	Follow the instructions for washing times.
f. Incubation temperature is incorrect.	① Adhere to recommended incubation temperature. ② Avoid putting plates in variable environmental conditions.
g. The plate was not sealed with sealing film during incubation, resulting in volatile contamination of the solution.	Add plate sealing film for incubation.
h. The color development time is too long.	According to the actual color development, shorten or extend the time as appropriate, generally about 15 min, but not more than 30 min.
i. Microplate reader setting error.	Check the wavelength and filter Settings.

VI FAQs for ELISA

ELISA Kit

Q1 How many samples can the 96T test? Do I need to make a duplication?

A1 In order to ensure the accuracy of the experimental results, we recommend that both standard and sample are set with duplication, but it is not required that the ELISA experiment make a duplication/triple-well. You can set the duplication experiment according to your own needs. Detailed information can be found in the following table:

ELISA Kit	Standard Curve Parallel	Sample Parallel	Number of Samples Can be Made
96T	1	1	88
		2	44
		3	29
	2	1	80
		2	40
		3	26
	3	1	72
		2	36
		3	24

Q2 Can the standard in the kit be reused after dilution?

A2 As the standard substance itself is freeze-dried powder, its morphology will change after dissolution, and its concentration will decrease. Compared with the powder, its stability will reduced a lot. If it is directly dissolved and placed at room temperature until the whole experiment is finished, it cannot be used again.

If it is really necessary to do multiple experiments and there is a time interval between them, it is recommended to subpack the newly dissolved standard (2~3 pieces), use one pack for this test, and store the rest packs immediately at -20℃ (constant temperature), it would cause no obvious effect if use the rest packs within half a month.

Q3 Can components be mixed within/between kits? Can the sample diluent be replaced with saline /PBS?

A3 The diluents in the kit cannot be mixed. component in the different batches kits can't be mixed (except stop solution).

The kit comes with a special standard & sample diluent that can be used to dilute samples. If the sample diluent is missing, it is not recommended to replace it directly with saline /PBS. The two solvents are not perfectly matched with the kit system, which may affect the accuracy of the measured results.

Q4 The kit needs to be used for many times, but there are many samples. Is it possible to do the standard curve only once?

A4 Although the ELISA procedure is the same, the various influence factors of each experiment are changed, so the standard carve must be replot for each experiment in order to obtain more accurate and reliable detection results.

If multiple experiments are required, the highest concentration standard after dissolution can be stored at -20℃. Avoid repeated freezing and thawing, effective use within half a month (if the manual has additional emphasis on the storage and use of standard after dissolution, please refer to the requirements of the manual).

Q5 If there are many samples to be assayed, can the number of wells in the standard curve be reduced?

A5 In order to ensure the accuracy of the results, it is not recommended to reduce the number of wells in the standard curve, please ensure that you can assay at least 6 different standard concentrations (including blank wells).

Q6 Can the 96-well plate of kit be used at an interval of 3 months after the first use?

A6 Our kit is valid for half a year, after unpacking, you only need to store it according to the storage temperature on the instructions. If the kit is stored properly, it can be used after 3 months, but it should be noted that: when placing the reagent at 4℃, do not store it on the inner wall of the refrigerator, which may affect the activity of the reagent due to the frost freezing of the refrigerator.

If you need to perform multiple experiments, please remove the unused slats in advance, and place them in aluminum foil bags, sealed storage according to the temperature specified in the instructions.

Q7 The sample/reagent volume is not insufficient, can the sample volume be reduce uniformly?

A7 In the ELISA experiment, the loading volume of each step needs to strictly follow the instructions, otherwise, the experimental system will be changed, and the results will be inaccurate. If your sample volume is really not enough, please contact technical support in time to confirm whether the sample can be diluted, and judge the sample concentration through pre-experiment. If the reagent volume is insufficient, please reduce the number of Wells to be measured according to the actual volume, and cannot be diluted before detection.

Q8 How accurate and stable is the kit?

A8 The accuracy and stability of the kit are very excellent. All products go through a strict three-step inspection before delivery to ensure product quality. In addition, our company independently developed a special stabilizer for the kit, which can guarantee the activity of each component.

Q9 What is the difference between scientific research kit and clinical kit?

A9 [Industry supervision] Scientific research kits are not supervised by specialized agencies, and are managed by competent departments of science and technology and animal epidemic prevention. There is no special mandatory legal requirement, and the product does not need to be registered. Clinical kits are administered by the State Food and Drug Administration; there are very strict laws and regulations supervision system. Detection reagents need to go through clinical verification and obtain medical device registration certificate.

[Product application] The users of the scientific research kit are mainly scientific research workers, and the ultimate goal is to obtain experimental data and experimental conclusions in basic research. It requires the diversity of products (wide sample types, wide detection range, etc.), the accuracy of detection, the speed of product development and the improvement of technical services. Individual product batch is less, but the product variety is wide. Clinical kits belong to applied research, which has more stringent requirements for the safety and effectiveness of research results. Because it directly involves human health, the indicators are less but the product standardization is high, and the Individual product batch is more.

Q10 How long is the shelf life of the kit? How long is the delivery time? How to transport?

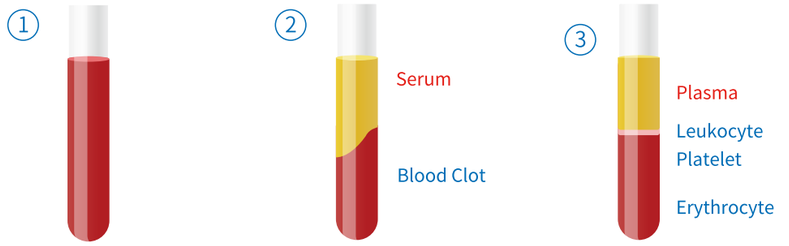
A10 The kit is valid for 12 months; the delivery time is 2~3 working days, and the delivery is delivered with SF ice packs to ensure stable 4°C transportation conditions.

Sample

Q1 What's the difference between serum and plasma?

A1 According to different conditions of collection, whole blood is divided into two types: non-anticoagulant and anticoagulant.

The upper yellow liquid separated with non-anticoagulation is called serum; the upper yellow liquid separated by anticoagulant is called plasma. The serum does not contain fibrinogen and certain coagulation factors.



Q2 Which vacuum tube should be used to collect serum and plasma samples?

A2 Serum: choose a blood collection tube without anticoagulants or a blood collection tube with anticoagulants, and blood will coagulate.

According to the color of the tube cover, it is usually divided into:
Red: no additives, no anticoagulants;
Orange: contains coagulant;
Yellow: contains coagulant and inert separation gel.

Plasma: choose a blood collection tube with anticoagulant, blood will not clot.

According to the color of the tube cover, it is usually divided into:
Purple: EDTA-containing salt anticoagulant;
Green: heparin-containing anticoagulant.

Q3 How much blood can be collected from rats and mice?

A3 According to the requirements of the blood collection amount required by the experimental protocol, we can choose different blood collection methods.

Blood Collection Method	Suitable Animal	Blood Collection Volume（mL）	Number of Blood Collection	Operation Difficulty	Application
Blood collection from the tail	Mouse	0.1~0.2	Many times	easier	Suitable for small amount of blood collection, less damage
	Rat	0.2~0.4			
Blood collection from orbital venous plexus	Mouse	0.2~0.3	Many times	easy	Suitable for multiple blood collection, less damage
	Rat	0.4~0.6			
Blood collection from eyeballs	Mouse	0.6~1.0	Not many times	easier	Executed after blood collection, more damage
	Rat	Less use			
Blood collection from heart	Mouse	0.5~0.8	Not many times	harder	Suitable for end-stage blood collection, great damage
	Rat	1.0~1.5			
Blood collection by decapitation	Mouse	0.8~1.2	Once	easier	Suitable for end-stage blood collection, great damage
	Rat	3.0~8.0			

Q4 How to avoid hemolysis?

A4 Hemolysis can be caused by various physicochemical factors and toxins. In vitro, mechanical strong oscillation, low temperature freezing and other inducements can cause hemolysis. In order to avoid the influence of hemolysis on the detection results, attention should be paid to:

(1) When venous blood collection, the withdrawal pressure should not be too large;
(2) The one-time blood collection volume should not be too small;
(3) The whole blood collected should not be violently oscillated;
(4) The centrifugal speed should not be too high;
(5) The collected whole blood should be processed into serum/plasma as soon as possible, and the whole blood cannot be freeze-thawed;
(6) If anesthesia is required for blood collection, an anesthetic with no hemolytic effect should be used.

Q5 How to deal with high fat samples?

A5 The high-fat sample contains fat and is not a homogeneous solution. If the sample is directly loaded, it will affect the binding of antigens and antibodies, resulting in inaccurate measurement values. It is recommended to centrifuge at a high speed (5000×g) first, stand for 10 min, take the clarified liquid in the middle layer, and then centrifuge at a high speed (≥5000×g) once to take the middle and lower layers for detection.

Q6 The methods of "repeated freeze-thaw" when collecting tissue or cell samples:

A6 Tissue Sample: After finishing tissue grinding, place it at -80°C for 1 h/ liquid nitrogen for 0.5 h, and then gently shake it in a water bath at 30°C to melt it quickly. Repeat this operation 1~2 times.

Cell Sample: Repeat the above freeze-thaw operation 2~3 times. If it is a membrane protein, it can be appropriately sonicated, but the temperature and frequency of sonicator need to be controlled.

It is recommended to add protease inhibitors to the sample in advance. PMSF is generally recommended.

Q7 The methods of "ultrasonic processing" when collecting tissue or cell samples:

A7 Using ultrasonic cell crusher, ultrasonic treatment of the suspension to lyse the cells (reference: Soniprep150 ultrasonic generator, ultrasonic treatment of 30 s at an amplitude of 14 μm, cell fragmentation; Or use an ultrasonic crusher, 200 W, 2 s/ times, gap 3 s, total time 3~5 min or 400 amp, 5 s/times, gap 10 s, repeated 3~5 times), and then centrifuge at 2 to 8°C at 1500×g for 10 min, remove the cell debris, collect the supernatant. Attention should be paid to temperature during ultrasound.

Q8 Can commercial/homemade lysates be used?

A8 It's not recommend to use commercial/homemade lysates.
The composition of different products is different, and the lysis capacity is also different, if the reagent contains surfactants such as SDS, it will affect the natural conformation of the target protein, and there may be a significant decrease in the measured value or false negative. In addition, due to the newly introduced solvent, the uncertain matrix interference may cause the background to rise, thus affecting the accuracy of the measurement value.

Q9 How do I determine if my sample needs to be diluted?

A9 The detection range of the kit is not equivalent to the concentration range of the substance to be tested in the sample. It is suggested to estimate the concentration of the substance to be measured in the sample through relevant literature before the experiment, and determine the actual concentration of the sample through pre-experiment.

If it is a routine sample type, you can consult ELISA technical support to obtain the recommended dilution of the sample and arrange pre-experiments for detection. If the concentration of the substance to be measured in the sample is too high or too low, dilute or concentrate the sample appropriately. If the concentration of analyte in the sample is much lower than the minimum detection limit of the kit, it is recommended to choose a more sensitive kit for detection.

Q10 What is the recommended method of sample storage?

A10 After collection and processing, if the sample is detected within 1 week, it can be stored at 2~8°C. If it cannot be detected in time, please aliquot according to the amount of one time and freeze it at -20°C(detected within 1 month) or -80°C(detected within 3 months) to avoid repeated freezing and thawing. Before detection, the frozen sample should be slowly melted and centrifuged to remove the sediment generated during the freeze-thaw process. Mix well at room temperature before use.

Q11 Are inflammatory factors used to detect cell extracts or cell supernatants?

A11 Cell extract and cell supernatant are generally detectable, it depends on your experimental purpose and research content. However, the components of the cell extract sample are complex and the preparation is complicated, so it is recommended that the detection of cell supernatant should be given priority.

Data Processing

Q1 What fitting function does the standard curve use? Can Excel be used to make standard curve? Why do I make the standard curve is not a straight line?

A1 It is recommended to use Origin software or other software with 4-PL (four-parameter logistic) function model to fit the standard curve. The 4-PL fitting method can reflect the linear relationship between concentration and absorbance more accurately, so as to obtain the concentration value of the substance to be measured in the sample more accurately. The Excel fitting methods are generally linear and polynomial, and these two fitting equations are not recommended as the fitting methods for ELISA.

Q2 How much is the R^2 of the ELISA standard curve considered qualified?

A2 R^2 can judge the reliability of the fitted standard curve, and the standard curve with $R^2 > 0.99$ can ensure the accuracy of the sample calculation results. If the fitting linearity of the standard curve is affected by individual standard curve wells due to operational errors, and individual standard curve wells can be reduced/eliminated before fitting, which will not significantly affect the calculation of sample results.

Q3 Can the standard curve in the instructions be used directly for calculation? What is the reason for the difference between the OD value and the image of my actual standard carve and the instructions?

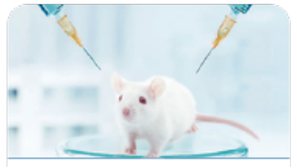
A3 The standard curve on the instruction is an indication standard curve, which is mainly used to show the shape of the standard curve after fitting and the quality control range of the highest/lowest OD value, and cannot be used directly. In addition, due to the influence of factors such as experimental operation, experimental environment and instrument parameter setting, the OD value you actually do may not be completely consistent with the instruction (overall high or low). In this case, it is recommended to control the color gradient of the first 4 blue wells in the color development stage, and the maximum OD value of the standard curve is above 1.2, and the correlation coefficient of the standard curve is above 0.99, which can be used as an effective standard curve.

If you have any after-sale inquiry or technical problems with Elabscience® products, you can contact us by phone(**1-888-852-8623** or **1-832-243-6086**) or email(**techsupport@elabscience.com**) for consultation (it is recommended to take photos of the color development results and keep the experimental data, the strips used and unused reagents). Technical support team 24 h online service, only to help you successfully complete the experiment.

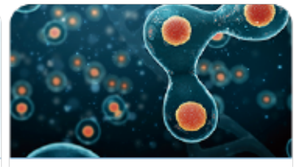
Other Technical Manuals

Technology Platform

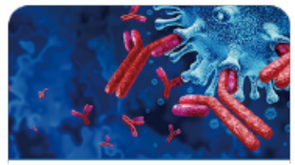
Protein Expression and Purification Platform



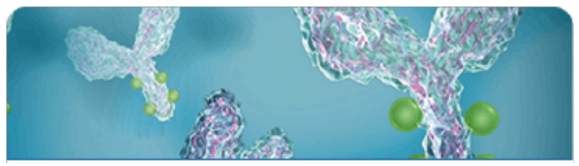
Animal Model Platform



Cell Biology Platform



Immunology R&D Platform



Pathology Platform

Molecular Biology Platform

POCT Platform