

Mouse Gliadin-IgE (Gliadin Immunoglobulin E) ELISA Kit

Cat #: orb2666963 (manual)

For research use only. Not intended for diagnostic use.

Product Features

Application	In vitro quantitative determination of Gliadin IgE concentrations in serum, plasma, cell culture supernatant and other biological samples.		
Reactivity	Mouse	Detection Method	Capture ELISA
Range	0.781-50ng/ml	Sensitivity	0.469ng/ml
Detection Duration	3 hours (excluding balancing and sample preparation)		
Samples needed for single well (Max)	Serum: 50ul, Plasma: 50ul, Cell Culture Supernatant: 100ul, cell or tissue lysate: 100ul, Other liquid samples: 50ul		
Specificity	Specifically recognize Gliadin IgE, no obvious cross reaction with other analogues		
Storage	2-8°C (for sealed box), please do not freeze!		

Internal Test Data: N/A

Assay Principle

This kit was based on capture enzyme-linked immune-sorbent assay technology. Anti Mouse IgE was pre-coated onto the 96-well plate. The HRP conjugated antigen was used as the detection antigen. The standards and pilot samples were added to the wells subsequently. After incubation, unbound conjugates were removed by wash buffer. Then, HRP detection antigen was added to bind with Gliadin IgE conjugated on Anti Mouse IgE. After washing, TMB substrates were added to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that turned yellow after adding acidic stop solution. Read the O.D. absorbance at 450nm in a microplate reader. The concentration of Gliadin IgE in the sample is positively correlated with OD450 and can be calculated by plotting the standard curve.

Kit Components

An unopened kit can be stored at 2-8°C for 6 months. If the opened kit is not used up, store the items separately according to the following conditions.

Item	Size(48T)	Size(96T)	Storage Condition for Opened Kit
ELISA Microplate(Dismountable)	8×6	8×12	Put the rest strips into a sealed foil bag with the desiccant. Stored for 1 month at 2-8°C; Stored for 12 months at -20°C
Lyophilized Standard	1vial	2vial	Put the rest of the standards into a desiccant bag. Stored for 1 month at 2-8°C; Stored for 12 months at -20°C
HRP-labeled Antigen (Concentrated, 100X)	60ul	120ul	2-8°C (Avoid Direct Light)
TMB Substrate	5ml	10ml	
Sample Dilution Buffer	10ml	20ml	2-8°C
Antigen Dilution Buffer	5ml	10ml	
Stop Solution	5ml	10ml	
Wash Buffer(25X)	15ml	30ml	
Plate Sealer	3 pieces	5 pieces	
Product Description	1 copy	1 copy	

Note: The liquid reagent bottle contains slightly more reagent than indicated on the label. Please use pipette accurately measure and do proportional dilution.

Materials Required but Not Supplied

1. Microplate reader (wavelength: 450nm)
2. 37°C incubator (CO2 incubator for cell culture is not recommended.)
3. Automated plate washer or multi-channel pipette/5ml pipettor (for manual washing purpose)
4. Precision single (0.5-10µL, 5-50µL, 20-200µL, 200-1000µL) and multi-channel pipette with disposable tips (calibration is required before use.)
5. Sterile tubes and Eppendorf tubes with disposable tips
6. Absorbent paper and loading slot
7. Deionized or distilled water

Sample Preparation

Sample Collection

1. **Serum:** Allow whole blood to clot at room temperature for 2 hours or at 2-8°C overnight. Centrifuge for 20 minutes at 1,000 × g and collect the supernatant for immediate analysis. Alternatively, aliquot and store at -20°C or -80°C for future assays.
2. **Plasma:** EDTA-Na2/K2 is recommended as the anticoagulant. Centrifuge samples for 15 minutes at 1000×g 2-8°C within 30 minutes after collection. Collect the supernatant for immediate analysis. Or you

can aliquot the supernatant and store it at -20°C or -80°C for future assays. For other anticoagulant types and uses, please refer to the sample preparation guideline.

3. Tissue Samples: Tissue samples should be prepared as homogenates as follows:

3.1 Place the tissue on ice. Remove residual blood by washing with pre-cooled PBS (0.01 M, pH 7.4), then weigh the tissue.

3.2 Homogenize on ice using lysis buffer. The required volume depends on tissue weight; typically, **9 mL PBS per 1 g tissue** is appropriate. Protease inhibitors are recommended (e.g., **1 mM PMSF**).

3.3 Further process the homogenate using sonication or freeze–thaw cycles (keep samples cold during sonication; freeze–thaw cycles may be repeated twice).

3.4 Centrifuge for **5 minutes at $5,000 \times g$** . Collect the supernatant for immediate analysis or aliquot and store at -20°C or -80°C .

3.5 Measure total protein concentration using a BCA assay for data normalization. Total protein concentration for ELISA is typically **1–3 mg/mL**. Some tissues (e.g., liver, kidney, pancreas) may contain high endogenous peroxidase activity that can react with TMB and cause false-positive results. If needed, inactivate with **1% H_2O_2 for 15 minutes**, then repeat the assay.

Notes: PBS or mild RIPA buffer may be used. If you use RIPA buffer, adjust to **pH 7.3**. Avoid NP-40, Triton X-100, and DTT, as these may significantly inhibit assay performance. A recommended lysis buffer is **50 mM Tris, 0.9% NaCl, 0.1% SDS, pH 7.3**. You may prepare this buffer in-house or contact Biorbyt for purchasing options.

4. **Cell Culture Supernatant:** Centrifuge at **2,500 rpm at $2-8^{\circ}\text{C}$ for 5 minutes**, then collect the clarified supernatant for immediate analysis. Alternatively, aliquot and store at -80°C for future assays.

5. Cell Lysate

5.1 Suspension Cells

Centrifuge at **2,500 rpm at $2-8^{\circ}\text{C}$ for 5 minutes** and collect the cells. Add pre-cooled PBS and mix gently, then centrifuge again to pellet the cells. Add **0.5–1 mL** lysis buffer with protease inhibitor (e.g., PMSF, final concentration **1 mmol/L**). Lyse on ice for **30–60 minutes** or disrupt by sonication.

5.2 Adherent Cells

Remove the supernatant and wash the cells three times with pre-cooled PBS. Add **0.5–1 mL** lysis buffer with protease inhibitor (e.g., PMSF, final concentration **1 mmol/L**). Scrape cells using a cell scraper. Lyse the suspension on ice for **30–60 minutes** or disrupt by sonication.

5.3 During lysis, mix by pipetting or gently shaking the tube to ensure complete lysis. Viscosity is typically caused by genomic DNA and can be reduced by sonication on ice (3–5 mm probe, 150–300 W, 3–5 seconds per pulse, 30-second intervals; total sonication time 1–2 minutes).

5.4 After lysis/sonication, centrifuge at **10,000 rpm at $2-8^{\circ}\text{C}$ for 10 minutes**. Transfer the supernatant to a microcentrifuge tube for immediate analysis, or aliquot and store at -80°C .

Notes: Refer to the tissue sample notes above.

6. Other Biological Sample

Centrifuge samples for **15 minutes at 1,000 × g** at **2–8°C**. Collect the supernatant for immediate analysis, or aliquot and store at **–80°C** for future assays.

Recommended Sample Dilution Ratio

For guidance on sample dilution and background information, refer to the shipped instructions or contact Biorbyt.

Important: Matrix components in serum/plasma can affect assay performance. Serum/plasma samples should be diluted at least **1:2** with Sample Dilution Buffer before testing.

If a different dilution ratio is required, refer to the universal dilution guide below. Volumes are for a single well, multiply by the number of wells for duplicates.

- **1:2 dilution:** Add 60 µL sample + 60 µL diluent; mix gently.
- **1:5 dilution:** Add 24 µL sample + 96 µL diluent; mix gently.
- **1:10 dilution:** Add 12 µL sample + 108 µL diluent; mix gently.
- **1:20 dilution:** Add 6 µL sample + 114 µL diluent; mix gently.
- **1:50 dilution:** Add 3 µL sample + 47 µL 0.9% NaCl into 100 µL diluent; mix gently.
- **1:100 dilution:** Add 3 µL sample + 177 µL 0.9% NaCl into 120 µL diluent; mix gently.
- **1:1,000 dilution:** Two-step dilution: prepare a 1:50 dilution first (normal saline), then prepare a 1:20 dilution; mix gently.
- **1:10,000 dilution:** Two-step dilution: prepare a 1:100 dilution first (normal saline), then repeat; mix gently.
- **1:100,000 dilution:** Three-step dilution: prepare 1:50 and 1:20 dilutions (normal saline for the first two steps), then prepare a 1:100 dilution; mix gently.

Note: Use at least **3 µL** sample in each dilution step. Mix gently to avoid foaming.

Notes for Sample

1. Use disposable, endotoxin-free blood collection tubes. Avoid hemolyzed or lipemic samples.
2. Recommended storage: up to **5 days at 2–8°C**, up to **6 months at –20°C**, and up to **2 years at –80°C**. For longer storage, samples may be stored in liquid nitrogen.
3. Thaw frozen samples rapidly in a **15–25°C** water bath to minimize ice-crystal effects. After thawing, centrifuge to remove precipitates, then mix thoroughly.
4. The assay detection range does not necessarily match the native analyte concentration in all sample types. If concentration falls outside the range, dilute or concentrate on samples as needed.
5. A pilot test is recommended for uncommon sample types or models without reference data.

Recombinant proteins may not always be recognized by the kit antibodies, which may result in undetectable signal.

Reagent Preparation

Take the ELISA kit from the fridge around 20 minutes earlier and equilibrate to room temperature (18–25°C). For repeated assays, please just take the strips and standards required for the current assay, store the rest materials according to the relevant condition.

1. Wash Buffer

Dilute 30ml (15ml for 48T) concentrated wash buffer to 750ml (375ml for 48T) wash buffer with deionized or distilled water and mix well. (The recommended resistivity of ultrapure water is 18MΩ.) Alternatively, take appropriate amount of concentrated wash buffer according to the assay requirement, then create a 25-fold dilution and mix well. Store the rest solution at 2-8°C.

Crystals formed in the concentrated wash buffer can be heated by water bath at 40°C till complete dissolution. (Heating temperature should be below 50°C.) Mix well for the next step. It's better to use up the prepared wash buffer in one day. Store the rest buffer at 2-8°C within 48h.

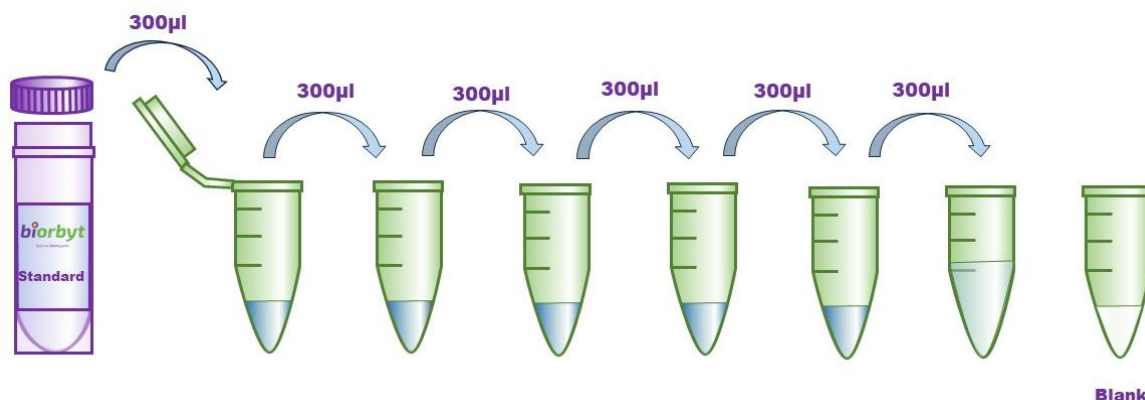
2. Standards

2.1. Centrifuge standards tube for 1min at 10000xg. Label it as Zero tube.

2.2. Add 1ml sample dilution buffer into the standard tube. Tighten the tube cap and let it stand for 2min at room temperature. Invert the tube several times to mix gently. (Or you can mix it using a low-speed vortex mixer for 3-5 seconds.)

2.3. Centrifuge the tubes for 1min at 1000xg, making the liquid towards the bottom of tube and removing possible bubbles.

2.4. Standard dilution: Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3ml of the sample dilution buffer into each tube. Add 0.3ml solution from zero tube into 1/2 tube and mix them thoroughly. Transfer 0.3ml from 1/2 tube into 1/4 tube and mix them thoroughly. Transfer 0.3ml from 1/4 tube into 1/8 tube and mix them thoroughly, so on till 1/64 tube. Now blank tube only contains 0.3ml sample dilution buffer. The standard concentration from zero tube to blank tube is 50ng/ml, 25ng/ml, 12.5ng/ml, 6.25ng/ml, 3.125ng/ml, 1.562ng/ml, 0.781ng/ml, 0ng/ml.



Notes: Store the zero tube with dissolved standards at 2-8°C and use it within 12h. Other diluted working solutions containing standards should be used in 2h.

3. Preparation of HRP-labeled Antigen Working Solution

The working solution should be prepared within 30min before the assay and can't be stored for a long time.

3.1. Calculate required total volume of the working solution: 100ul/well x quantity of wells. (It's better to prepare additional 100ul-200ul.)

3.2. Centrifuge for 1min at 1000xg in low speed and bring down the concentrated biotin-labeled antibody to the bottom of tube.

3.3. Dilute the HRP detection antigen with antigen dilution buffer at 1:99 and mix them thoroughly. (e.g. Add 10ul concentrated HRP-labeled antigen into 990ul antigen dilution buffer.)

Assay Procedure

Step 1: Wash plate 2 times before adding Standard, Sample and Control (blank) wells!

Step 2: Add 100ul standard or sample into each well, seal the plate and static incubate for 90 minutes at 37°C.

Washing: Wash the plate three times and immerse for 1min each time.

Step 3: Add 100ul HRP-labeled antigen working solution into each well, seal the plate and static incubate for 30 minutes at 37°C.

Washing: Wash the plate five times and immerse for 1min each time.

Step 4: Add 90ul TMB substrate solution, seal the plate and static incubate for 10-20 minutes at 37°C. (Accurate TMB visualization control is required.)

Step 5: Add 50ul stop solution. Read at 450nm immediately and calculate.

Detailed Assay Procedure

When diluting samples and reagents, they must be mixed completely. It's recommended to plot a standard curve for each test.

1. Set standard, pilot samples, control (blank) wells on the pre-coated plate respectively, and then, records their positions. It's recommended to measure each standard and sample in duplicate to decrease experimental errors. **Wash plate 2 times before adding Standard, Sample and Control (blank) wells!**
2. Standards and samples loading: Aliquot 100ul of zero tube, 1st tube, 2nd tube, 3rd tube, 4th tube, 5th tube, 6th tube into each standard well. Also add 100ul sample dilution buffer into the control (blank) well. Then, add 100ul pilot samples into each sample well. Seal the plate and static incubate for 90 minutes at 37°C. (Add the solution to the bottom of each well. Mix gently and without touch the sidewall and foam the sample.)
3. Wash three times: Remove the cover, then absorb the liquid in the plate or tap the plate on a clean absorbent paper two or three times. Add 350ul wash buffer into each well and immerse for 1min. Discard the liquid in the well and tap on the absorbent paper again. Repeat the washing step three times.
4. HRP-labeled Antigen: Add 100ul HRP-labeled antigen working solution into each well. Seal the plate and static incubate for 30 minutes at 37°C.
5. Wash five times: Remove the cover and then wash the plate with wash buffer five times. Read washing method in step 5.
6. TMB Substrate: Add 90ul TMB Substrate into each well, seal the plate and static incubate at 37°C in dark within 10-20 minutes. Run the microplate reader and preheat for 15min.
(Notes: Please do not use the reagent reservoirs used by HRP couplings. The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells. Weaker or stronger color intensity is unacceptable.)
7. Stop: Keep the liquid in the well after staining. Add 50ul stop solution into each well. The color will turn yellow immediately. The order for adding stop solution and TMB substrate solution is the same.
8. OD Measurement: Read the O.D. absorbance at 450nm in a microplate reader immediately. (If your microplate reader has a choice of correction wavelength, set it to 570nm or 630nm. Correct the read value to the OD450 value minus the OD570 or OD630 value. In this way, the OD value of non-chromogenic

substances can be corrected and removed, thus obtaining more accurate results. If the microplate reader does not have a 570nm or 630nm wavelength, the original OD450 value can be used.)

Calculation of Results

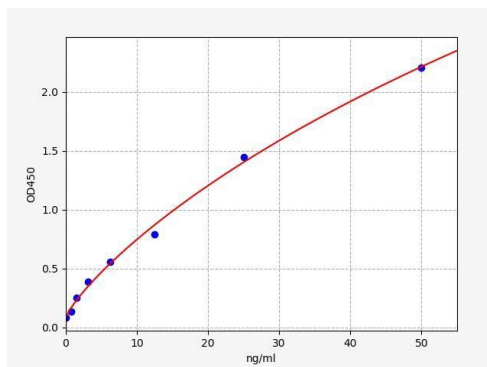
1. Calculate the mean OD450 value (using the original OD450 value or the corrected OD450 value) of the duplicate readings for each standard, control, and sample. Then, obtain the value of calculation by subtracting the OD450 blank.
2. Create a four-parameter logistic curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis. (Remove the OD450 blank during plotting.) Alternatively, you can use the curve fitting software offered by the microplate reader (e.g. Thermo SkanIt RE software, Curve Expert 1.3 or 1.4).
3. Calculate the sample concentration by substituting OD450 value into the standard curve. Diluted samples should be multiplied by the relevant dilution ratio.

Typical Data & Standard Curve

This product has been tested by Quality Control Department and meets performance specifications mentioned in the manual. (The humidity in the laboratory is 20%-60%, and the temperature is 18°C - 25°C. TMB was balanced to 37°C before color development and incubated at 37°C for 15 minutes in the dark after adding the enzyme label plate holes.)

The following assay data are provided for reference, since experimental environment and operation are different. The establishment of standard curve depends on your own assay.

STD.(ng/ml)	OD-1	OD-2	Average	Corrected
0	0.082	0.084	0.083	0
0.781	0.13	0.134	0.132	0.049
1.562	0.246	0.254	0.25	0.167
3.125	0.384	0.396	0.39	0.307
6.25	0.548	0.564	0.556	0.473
12.5	0.783	0.805	0.794	0.711
25	1.428	1.47	1.449	1.366
50	2.174	2.238	2.206	2.123



Performance

Precision

Intra-assay Precision: samples with low, medium and high concentration are tested 20 times on same plate.

Inter-assay Precision: samples with low, medium and high concentration are tested 20 times on three different plates.

Item	Intra-assay Precision			Inter-assay Precision		
	1	2	3	1	2	3
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (ng/ml)	1.49	6.18	24.48	1.64	6.39	25.04
Standard deviation	0.06	0.37	1.4	0.1	0.33	1.08
CV(%)	4.36	5.92	5.72	5.95	5.23	4.32

Recovery

Add a certain amount of Gliadin IgE into the sample. Calculate the recovery by comparing the measured value with the expected amount of Gliadin IgE in the sample.

Matrix	Recovery Range (%)	Average (%)
Serum(n=5)	85-95	90
EDTA Plasma(n=5)	86-103	94
Heparin Plasma(n=5)	87-103	95

Linearity

Dilute the sample with a certain amount of Gliadin IgE at 1:2, 1:4 and 1:8 to get the recovery range.

Sample	1:2	1:4	1:8
Serum(n=5)	85-105%	85-97%	91-105%

EDTA Plasma(n=5)	84-101%	84-98%	83-94%
Heparin Plasma(n=5)	80-93%	85-93%	83-98%

Stability

Perform the stability test for the sealed kit at 37°C and 2-8°C and get relevant data.

ELISA kit(n=5)	37°C for 1 month	2-8°C for 6 months
Average (%)	80	95-100

ELISA Troubleshooting

High background/non-specific staining

Description of results	Possible reason	Recommendations and precautions
After termination, the whole plate results show a uniform yellow or light color; or the Standard curve is linear but the background is too high	The yellowing of the whole plate may be caused by wrong addition of other reagents	Check the components and lot numbers of the reagents before the experiment, and confirm that all components belong to the corresponding kit. Reagents from different kits or different lot numbers cannot be mixed.
	ELISA plate was not washed sufficiently	Make sure that the same amount of Washing Solution is added to each microwell during the washing process. After washing, press the ELISA plate firmly on the absorbent paper to remove the residual buffer.
	Incubation time too long	Please strictly follow the steps of the manual
	Streptavidin-HRP contaminates the tip and TMB container or positive control contaminates the Pre-coated Microplate	When absorbing different reagents, the tips should be replaced. When configuring different reagent components, different storage vessels should be used. Please use a pipette during operation.
	Biotinylated Antibody or Streptavidin-HRP concentration too high	Check whether the concentration calculation is correct or use after further dilution.
	Substrate exposure or contamination prior to use	Store in the dark at all times before adding substrate.
	Color development time is too long	Please strictly follow the steps of the manual.
	The wrong filter was used when the absorbance value was read	When TMB is used as the substrate, the absorbance should be read at 450 nm.

NO color plates

Description of results	Possible reason	Recommendations and precautions
After the color development step, all wells of the ELISA plate are colorless; the positive control is not obvious	Mixed use of component reagents	Please read labels clearly when preparing or using
	In the process of plate washing and sample enzyme contaminated addition, the marker is and inactivated, and loses its ability to catalyze the color developing agent	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3 , etc.) and confirm that the container for preparing the Wash Solution has been washed.
	Missing a reagent or a step	Review the manual in detail and strictly follow the operating steps

Light color

Description of results	Possible reason	Recommendations and precautions
The Standard is normal, the color of the sample is light	The sample uses NaN_3 preservative, which inhibits the reaction of the enzyme	Samples cannot use NaN_3
	The sample to be tested may not contain strong positive samples, so the result may be normal	In case of doubt, please test again.
The visual result is normal, but the reading value of the microplate reader is low	Wrong filter used for absorbance reading	When TMB is used as the substrate, the absorbance should be read at 450 nm.
All wells, including Standard and Samples, are lighter in color	Insufficient incubation time	Timer accurate timing
	Insufficient color reaction	Usually 15 - 30 minutes
	The number of washings increases, and the dilution ratio of the concentrated lotion does not meet the requirements	Reduce the impact of washing, dilute the concentrated lotion and washing time according to the manual, and accurately record the washing times and dosage.
	Distilled water quality problem	The prepared lotion must be tested to see if the pH value is neutral.
	In the process of plate washing and sample addition, the enzyme marker is contaminated and inactivated, and loses its ability to catalyze the color developing agent.	Confirm that the container holding the ELISA plate does not contain enzyme inhibitors (such as NaN_3 , etc.), confirm that the container for preparing the Washing Solution has been washed, and confirm that the purified water for preparing the Washing Solution meets the requirements and is not contaminated.

	The kit has expired or been improperly stored	Please use it within the expiration and store it in accordance with the storage conditions recommended in the manual to avoid contamination.
	Reagents and samples are not equilibrated before use	All reagents and samples should be equilibrated at room temperature for about 30 minutes.
	Insufficient suction volume of the pipette, too fast discharge of pipetting suction, too much liquid hanging on the inner wall of the tip or the inner wall is not clean.	To calibrate the pipette, the tips should be matched, each time the tips should fit tightly, the pipetting should not be too fast, and the discharge should be complete. The inner wall of the tips should be clean, and it is best to use it once.
Poor repeatability	Incubation temperature constant temperature effect is not good	Keep the temperature constant to avoid the local temperature being too high or too low
	When adding liquid, too much remains on the medial wall of wells	When adding liquid, the tip should try to add liquid along the bottom of the medial wall of wells without touching the bottom of the hole.
	Reuse of consumables	The tips should be replaced when different reagents are drawn, and different storage vessels should be used when configuring different reagent components.
	The bottom of the microwell is scratched or there is dirt	Be careful when operating, be careful not to touch the bottom and wipe the bottom of the microplate to remove dirt or fingerprints. Technical repetition of the same sample for 3 times, including more than 2 approximate values.
	Cross-contamination during sample addition	Try to avoid cross-contamination when adding samples
The color of plate is chaotic and irregular	Cross-contamination from manual plate washing	When washing the plates by hand, the first 3 injections of the lotion should be discarded immediately, and the soaking time should be set for the next few times to reduce cross-contamination.
	Cross-contamination when clapping	Use a suitable absorbent paper towel when clapping the plate, do not pat irrelevant substances into the well of the plate, and try not to pat in the same position to avoid cross-contamination.

Description of results	Possible reason	Recommendations and precautions
------------------------	-----------------	---------------------------------

The color of plate is chaotic and irregular	The liquid filling head of the plate washer is blocked, resulting in unsatisfactory liquid addition or large residual amount of liquid suction, resulting in the color of plate is chaotic and irregular	Unblock the liquid addition head, so that each well is filled with washing liquid when washing the plate and the residual amount should be small when aspirating liquid.
	Incomplete centrifugation of the sample, resulting in coagulation in the reaction well or interference of sediment or residual cellular components	Serum plasma should be fully centrifuged at 3000 rpm for more than 6 minutes
	The sample is stored for too long time, resulting in contamination.	Samples should be kept fresh or stored at low temperature to prevent contamination
	Incorrect preparation of Washing Solution or direct misuse of concentrated Washing Solution	Please configure according to the manual

Declaration

1. Limited to current conditions and scientific techniques, all raw materials are not completely identified and analyzed. This product may have a technology-related quality risk.
2. During the ELISA kit development, some endogenous interferons (not all) in the biological sample have been removed or decreased.
3. The final assay result is related to the validity of reagents, experimental operation and environment. We are only responsible for this kit, excluding sample consumption during using this kit. Before use, please consider and prepare enough samples required by the assay.
4. To get a satisfied assay result, please use all reagents offered by this kit. Don't use any product from other vendors. Strictly follow instructions of this manual.
5. During assay procedure, incorrect reagents preparation and parameter setting of the microplate reader may result in the abnormal result. Before assay, please read this manual carefully and adjust instruments.
6. Even if the assay is performed by the same person, results in two independent assays may be different. Thus, each step in the assay should be controlled to ensure the reproducibility.
7. Before delivery, this kit is subject to the strict QC. Influenced by transportation conditions and experimental devices, the assay result got by the customer may be different from original data. Inter-assay CV between different batches may be caused by reasons before.
8. This kit is not compared to similar kits from other vendors or methods for testing the same detection target. Thus, assay results may be inconsistent.
9. This kit allows for research use only. For IVD or other purposes, we are not responsible for relevant consequences and doesn't bear any legal liability.

Precautions for Kits

1. When using different ELISA kits, labelling is required to avoid mixed components and failed assay.

2. After opening the kit, please refer to the table of storage condition for coated plate and standards (Dampness may decrease the activity.). If any component is missing or damaged during the assay or storage, please contact us for ordering a new one to replace. (e.g. lyophilized standard)
3. Sterile and disposable tips are required during the assay. After use, the reagents bottle cap has to be tightened to avoid the microbial contamination and evaporation.
4. While manual washing, please keep tips or pipettors for adding wash buffer away from the well. Insufficient washing or contamination easily causes false positive and high background.
5. During the assay, prepare required reagents for next step in advance. After washing, add the reagent into the well in time to avoid dryness. Otherwise, dry plate will result in the failed assay.
6. Before confirmation, reagents from other batches or sources should not be used in this kit.
7. Don't reuse tips and tubes to avoid cross contamination.
8. After loading, seal the plate to avoid the evaporation of the sample during incubation. Complete the incubation process at recommended temperature.
9. Please wear the lab coat, mask and gloves to protect yourself during the assay. Especially, for the detection of blood or other body fluid samples, please follow regulations on safety protection of biological laboratory.