

## Human OPA1 (Optic Atrophy 1) ELISA Kit

Cat #: orb1714720 (manual)

*For research use only. Not intended for diagnostic use*

### Product Features

|                                      |                                                                                                                                     |                  |            |
|--------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------|------------------|------------|
| Application                          | In vitro quantitative determination of OPA1 concentrations in serum, plasma, cell culture supernatant and other biological samples. |                  |            |
| Reactivity                           | Human                                                                                                                               | Detection Method | Sandwich   |
| Range                                | 31.25-2000pg/ml                                                                                                                     | Sensitivity      | 18.75pg/ml |
| Detection Duration                   | 4 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 OPA1, no obvious cross reaction with other analogues                                                         |                  |            |
| Storage                              | 2-8°C (for sealed box), please do not freeze!                                                                                       |                  |            |

### Background

Uniprot ID: O60313

Optic Atrophy-1, aka Dynamin-like 120 kDa protein, mitochondrial, is a Dynamin-related GTPase required for mitochondrial fusion and regulation of apoptosis. OPA1 exists as a single-pass membrane protein in the mitochondrion inner membrane as well as in soluble forms in mitochondrion intermembrane space, and is expressed in retina, brain, testis, heart, skeletal muscles. Human OPA1 binds PARL and interacts with CHCHD3 as well as IMMT. Proteolytic processing in response to intrinsic apoptotic signals may lead to disassembly of OPA1 oligomers and release of the caspase activator cytochrome C into mitochondrial intermembrane space. Defects in OPA1 have been linked to optic atrophy type 1 and dominant optic atrophy plus syndrome.

### Internal Test Data

| Sample Type        | Recommended Dilution Ratio | Content                 |
|--------------------|----------------------------|-------------------------|
| Healthy serum      | 1/2 dilution               | 180-1260pg/ml           |
| HepG2 cell lysates | 1/2 dilution               | 526pg/mg(total protein) |

### Assay Principle

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Anti OPA1 antibody was pre-coated onto the 96-well plate. The biotin conjugated anti OPA1 antibody was used as the detection antibody. The standards and pilot samples were added to the wells subsequently. After incubation, unbound conjugates were removed by wash buffer. Then, biotinylated detection antibody was added to bind with OPA1 conjugated on coated antibody. After washing off unbound conjugates, HRP-Streptavidin was added. After a third 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 a stop solution. Read the O.D. absorbance at 450nm in a microplate reader. The concentration of OPA1 in the sample was calculated by drawing a standard curve. The concentration of the target substance is proportional to the OD450 value.

### Kit Components

An unopened kit can be stored at 2-8°C for 12 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                         | 1 vial    | 2 vials   | Put the rest standards into a desiccant bag. Stored for 1 month at 2-8°C; Stored for 12 months at -20°C                   |
| Biotin-labeled Antibody (Concentrated, 100X) | 60ul      | 120ul     | 2-8°C (Avoid Direct Light)                                                                                                |
| HRP-Streptavidin Conjugate (SABC, 100X)      | 60ul      | 120ul     |                                                                                                                           |
| TMB Substrate                                | 5ml       | 10ml      |                                                                                                                           |
| Sample Dilution Buffer                       | 10ml      | 20ml      | 2-8°C                                                                                                                     |
| Antibody Dilution Buffer                     | 5ml       | 10ml      |                                                                                                                           |
| SABC 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 (CO<sub>2</sub> 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 and Storage

##### 1. Serum

Place whole blood sample at room temperature for 2 hours or at 2-8°C overnight. Centrifuge for 20min at 1000xg and collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

##### 2. Plasma

EDTA-Na<sub>2</sub>/K<sub>2</sub> is recommended as the anticoagulant. Centrifuge samples for 15 minutes at 1000×g 2-8°C within 30 minutes after collection. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay. For other anticoagulant types and uses, please refer to the sample preparation guideline.

##### 3. Tissue Sample

Generally, tissue samples are required to be made into homogenization. Protocol is as below:

3.1. Place the target tissue on the ice. Remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Then weigh for usage.

3.2. Use lysate to grind tissue homogenates on the ice. The adding volume of lysate depends on the weight of the tissue. Usually, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS (e.g. 1mM PMSF).

3.3. Do further process using ultrasonic disruption or freeze-thaw cycles (Ice bath for cooling is required during ultrasonic disruption; Freeze-thaw cycles can be repeated twice.) to get the homogenates.

3.4. Homogenates are then centrifuged for 5 minutes at 5000×g. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future's assay.

3.5. Determine total protein concentration by BCA kit for further data analysis. Usually, total protein concentration for ELISA assay should be within 1-3mg/ml. Some tissue samples such as liver, kidney, pancreas which containing a higher endogenous peroxidase concentration may react with TMB substrate causing false positivity. In that case, try to use 1% H<sub>2</sub>O<sub>2</sub> for 15min inactivation and perform the assay again.

Notes: PBS buffer or the mild RIPA lysis can be used as lysates. While using RIPA lysis, make the PH=7.3. Avoid using any reagents containing NP-40 lysis buffer, Triton X-100 surfactant, or DTT due to their severe inhibition for kits' working. We recommend using 50mM Tris+0.9%NaCL+0.1%SDS, PH7.3. You can prepare by yourself or contact us for purchasing.

#### 4. Cell Culture Supernatant

Collect the supernatant: Centrifuge at 2500 rpm at 2-8°C for 5 minutes, then collect clarified cell culture supernatant to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

#### 5. Cell Lysate

5.1. Suspension Cell Lysate: Centrifuge at 2500 rpm at 2-8°C for 5 minutes and collect cells. Then add pre-cooling PBS into collected cell and mix gently. Recollect cell by repeating centrifugation. Add 0.5-1ml cell lysate and appropriate protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Lyse the cell on ice for 30min-1h or disrupt the cell by ultrasonic disruption.

5.2. Adherent Cell Lysate: Absorb supernatant and add pre-cooling PBS to wash three times. Add 0.5-1ml cell lysate and appropriate protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Scrape the adherent cell with cell scraper. Lyse the cell suspension added in the centrifuge tube on ice for 30min-1h or disrupt the cell by ultrasonic disruption.

5.3. During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. Mucilaginous product is DNA which can be disrupted by ultrasonic cell disruptor on ice. (3~5mm probe, 150-300W, 3~5 s/time, 30s intervals for 1~2s working).

5.4. At the end of lysate or ultrasonic disruption, centrifuge at 10000rpm at 2-8°C for 10 minutes. Then, the supernatant is added into EP tube to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

Notes: Read notes in tissue sample.

#### 6. Other Biological Sample

Centrifuge samples for 15 minutes at 1000×g at 2-8°C. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -80°C for future's assay.

Recommended reagents for sample preparation: 100mM PMSF protease inhibitor, Lysis Buffer (for ELISA).

## Recommended Sample Dilution Ratio

Please refer to the following table of recommended dilution ratio for limited samples for reference.  
(ND: Not Detected)

| Sample Type        | Recommended Dilution Ratio | Content                 |
|--------------------|----------------------------|-------------------------|
| Healthy serum      | 1/2 dilution               | 180-1260pg/ml           |
| HepG2 cell lysates | 1/2 dilution               | 526pg/mg(total protein) |

**The matrix components in serum/plasma will affect the test results, which it need to be diluted at least 1/2 with Sample Dilution Buffer before testing!**

If other dilution ratio for your sample model is required, please refer to the universal dilution ratio below.  
(The ratio is suitable for single-well assay. For duplicate assay, please follow the calculation: volume of sample and diluent x number of duplicate well)

For 2 fold dilution (1/2): One step dilution. Add 60μL sample into 60μL sample diluent and mix gently.

For 5 fold dilution (1/5): One step dilution. Add 24μL sample into 96μL sample diluent and mix gently.

For 10 fold dilution (1/10): One step dilution. Add 12μL sample into 108μL sample diluent and mix gently.

For 20 fold dilution (1/20): One step dilution. Add 6μL sample into 114μL sample diluent and mix gently.

For 50 fold dilution (1/50): One step dilution. Add 3μL sample and 47μL normal saline (0.9% NaCl) into 100 μL sample diluent and mix gently.

For 100 fold dilution (1/100): One step dilution. Add 3μL sample and 177μL normal saline into 120μL sample diluent and mix gently.

For 1000 fold dilution (1/1000): Two step dilution. Create a 50-fold dilution first (normal saline is used throughout the dilution). Then, create a 20-fold dilution and mix gently.

For 10000 fold dilution (1/10000): Two step dilution. Create a 100-fold dilution first (normal saline is used throughout the dilution). Then, create the same dilution again and mix gently.

For 100000 fold dilution (1/100000): Three step dilution. Create a 50-fold dilution and 20-fold dilution respectively (normal saline is used in the first two steps.) Finally, create a 100-fold dilution and mix gently.

**Notes: The volume in each dilution is not less than 3μL. Dilution factor should be within 100-fold. Mixing during dilution is required to avoid foaming.**

### ■ Note for Sample

1. Blood collection tubes should be disposable and non-endotoxin. Avoid using hemolyzed and lipemia samples.

2. The best sample storage condition: less than 5 days at 2-8°C; within 6 months at -20°C; within 2 years at -80°C. Stored in liquid nitrogen for a longer storage. When melting frozen samples, rapid water bath at 15-25°C can decrease the effect of ice crystal (0°C) on the sample. After melting, centrifuge to remove the precipitate, and then mix well.

3. The detection range of this kit is not equivalent to the concentration of analyze in the sample. For analyses with higher or lower concentration, please properly dilute or concentrate the sample.
4. Pretest is recommended for special samples without reference data to validate the validity.
5. Recombinant protein may not match with the capture or detection antibody in the kit, resulting in the undetectable assay.

## 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.

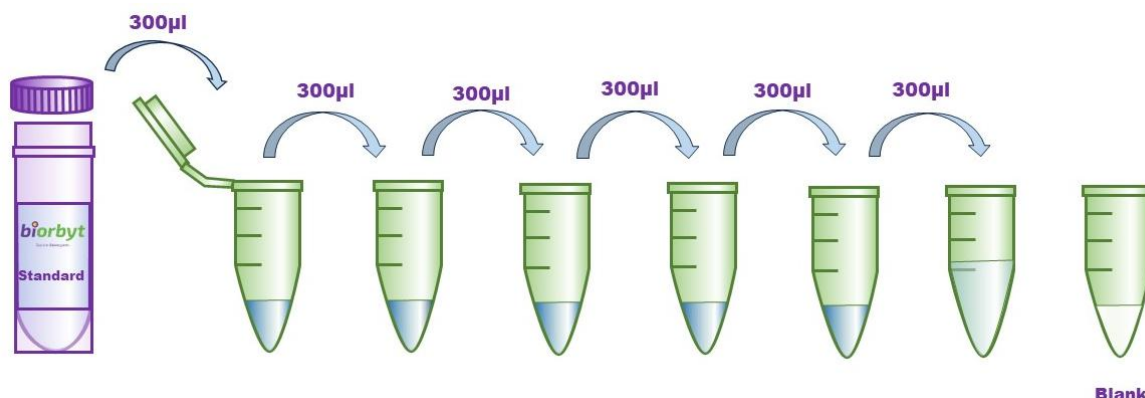
### 1. Wash Buffer

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 2000pg/ml, 1000pg/ml, 500pg/ml, 250pg/ml, 125pg/ml, 62.5pg/ml, 31.25pg/ml, 0pg/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 Biotin-labeled Antibody 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 biotinylated detection antibody with antibody dilution buffer at 1:99 and mix them thoroughly. (e.g. Add 10ul concentrated biotin-labeled antibody into 990ul antibody dilution buffer.)

### **4. Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution**

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

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

4.2. Centrifuge for 1min at 1000xg in low speed and bring down the concentrated SABC to the bottom of tube.

4.3. Dilute the concentrated SABC with SABC dilution buffer at 1:99 and mix them thoroughly. (e.g. Add 10ul concentrated SABC into 990ul SABC dilution buffer.)

### **Assay Procedure**

**Step 1:** Add 100ul standard or sample into each well, seal the plate and static incubate for 90 minutes at 37°C.

Washing: Wash the plate twice without soaking.

**Step 2:** Add 100ul biotin-labeled antibody working solution into each well, seal the plate and static incubate for 60 minutes at 37°C.

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

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



Washing: Wash the plate five times and soak 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.
2. Standards and samples loading: Aliquot 100ul of zero tube, 1<sup>st</sup> tube, 2<sup>nd</sup> tube, 3<sup>rd</sup> tube, 4<sup>th</sup> 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 twice: 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 without soaking. Discard the liquid in the well and tap on the absorbent paper again. Repeat the washing step twice.
4. Biotin-labeled Antibody: Add 100ul biotin-labeled antibody working solution into each well. Seal the plate and static incubate for 60 minutes at 37°C.
5. 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 soak for 1min. Discard the liquid in the well and tap on the absorbent paper again. Repeat the washing step three times.
6. HRP-Streptavidin Conjugate (SABC): Add 100ul SABC working solution into each well. Seal the plate and static incubate for 30 minutes at 37°C. (Put the whole bottle of TMB into the 37°C incubator to equilibrate for 30min.)
7. Wash five times: Remove the cover, and then wash the plate with wash buffer five times. Read washing method in step 5.
8. 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.)
9. 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.
10. 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

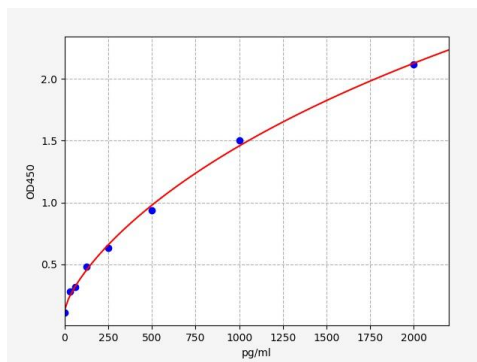
1. Calculate the mean OD450 value (using the original OD450 value or the corrected OD450 value) of the duplicate readings for each standard, control, and sample. Then, obtain the value of calculation by subtracting the OD450 blank.
2. Create a four-parameter logistic curve by plotting the mean absorbance 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.(pg/ml) | OD-1  | OD-2  | Average | Corrected |
|-------------|-------|-------|---------|-----------|
| 0           | 0.109 | 0.113 | 0.111   | 0         |
| 31.25       | 0.275 | 0.283 | 0.279   | 0.168     |
| 62.5        | 0.314 | 0.324 | 0.319   | 0.208     |
| 125         | 0.476 | 0.49  | 0.483   | 0.372     |
| 250         | 0.622 | 0.64  | 0.631   | 0.52      |
| 500         | 0.925 | 0.951 | 0.938   | 0.827     |
| 1000        | 1.481 | 1.523 | 1.502   | 1.391     |
| 2000        | 2.084 | 2.144 | 2.114   | 2.003     |



## 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               | Inter-assay Precision |       |       | Inter-assay Precision |       |       |
|--------------------|-----------------------|-------|-------|-----------------------|-------|-------|
| Sample             | 1                     | 2     | 3     | 1                     | 2     | 3     |
| n                  | 20                    | 20    | 20    | 20                    | 20    | 20    |
| Mean (pg/ml)       | 61.84                 | 268.1 | 982.6 | 59.51                 | 261.5 | 997.4 |
| Standard deviation | 3.09                  | 15.55 | 57.48 | 3.5                   | 15.95 | 61.54 |
| CV(%)              | 5                     | 5.8   | 5.85  | 5.88                  | 6.1   | 6.17  |

### Recovery

Add a certain amount of OPA1 into the sample. Calculate the recovery by comparing the measured value with the expected amount of OPA1 in the sample.

| Matrix              | Recovery Range (%) | Average (%) |
|---------------------|--------------------|-------------|
| Serum(n=5)          | 85-101             | 92          |
| EDTA Plasma(n=5)    | 92-104             | 99          |
| Heparin Plasma(n=5) | 96-101             | 99          |

### Linearity

Dilute the sample with a certain amount of OPA1 at 1:2, 1:4 and 1:8 to get the recovery range.

| Sample              | 1:2     | 1:4     | 1:8     |
|---------------------|---------|---------|---------|
| Serum(n=5)          | 85-102% | 91-101% | 85-98%  |
| EDTA Plasma(n=5)    | 91-98%  | 82-101% | 87-98%  |
| Heparin Plasma(n=5) | 87-102% | 88-99%  | 98-100% |

### 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 | 2-8°C for 12 months |
|----------------|------------------|--------------------|---------------------|
| Average (%)    | 80               | 95-100             | 85-98               |

## 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 | Mixed use of component reagents | Please read labels clearly when preparing or using |

|                                                |                                                                                                                                                                      |                                                                                                                                                                                                       |
|------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| colorless; the positive control is not obvious | 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 $\text{NaN}_3$ , 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 $\text{NaN}_3$ preservative, which inhibits the reaction of the enzyme                                                                                | Samples cannot use $\text{NaN}_3$                                                                                                                                                                                                                                                                                            |
|                                                                                    | 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 $\text{NaN}_3$ , 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.                                                                                                                 |
|                                             | Cross-contamination during sample addition                                                                                                                                | Technical repetition of the same sample for 3 times, including more than 2 approximate values.<br>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.