

## Mouse HS-IL-6(High sensitive Interleukin 6) Accquant® ELISA Kit

**Catalogue No.:** AQ-M0121-B

**Revision:** V 2.0

**Size:** 48T/96T

**Reactivity:** Mouse

**Range:** 6.25-400pg/ml

**Sensitivity:** 3.75pg/ml

**Application:** For quantitative detection of IL-6 in serum, plasma, tissue homogenates and other biological fluids.

**Storage:** 2-8°C

**Expiry Date:** see kit label

**Principle:** Sandwich

**NOTE: FOR RESEARCH USE ONLY.**

### Background

Interleukin 6 (IL-6) is a pleiotropic,  $\alpha$ -helical, 22-28 kDa phosphorylated and variably glycosylated cytokine that plays important roles in the acute phase reaction, inflammation, hematopoiesis, bone metabolism, and cancer progression. IL-6, along with TNF- $\alpha$  and IL-1, drives the acute inflammatory response. IL-6 is almost solely responsible for fever and the acute phase response in the liver, and it is important in the transition from acute inflammation to either acquired immunity or chronic inflammatory disease. Cells known to express IL-6 include CD8+ T cells, fibroblasts, synoviocytes, adipocytes, osteoblasts, megakaryocytes, endothelial cells (under the influence of endothelins), sympathetic neurons, cerebral cortex neurons, adrenal medulla chromaffin cells, retinal pigment cells, mast cells, keratinocytes, Langerhans cells, fetal and adult astrocytes, neutrophils, monocytes, eosinophils, colonic epithelial cells, and pancreatic islet beta cells. IL -6 production is generally correlated with cell activation and is normally kept in control by glucocorticoids, catecholamines, and secondary sex steroids.

### Principle of the Assay

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Capture antibody was precoated onto 96-well plates. And the biotin conjugated antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and

**Wuhan Fine Biotech Co., Ltd.**

1st Floor, Building A02, Optics Valley Biomedical Industrial Park, Wuhan, China (430075)

Tel: (0086)027-87384275

Fax: (0086)027-87800889

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washed with wash buffer. HRP-Streptavidin (HRP-SA) and Biotin System (BS) was added and unbound conjugates were washed away with wash buffer. Then by twice signal amplification, TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of target can be calculated.

### Kit Components

Item	Specifications(48T/96T)	Storage
ELISA Microplate(Dismountable)	8×6/8×12	2-8°C/-20°C
Lyophilized Standard	1vial/2vial	2-8°C/-20°C
Sample Dilution Buffer	5ml/10ml	2-8°C
Assay Dilution	2.5ml/5ml	2-8°C
Biotin-labeled Antibody	5ml/10ml	2-8°C(Avoid Direct Light)
HRP-Streptavidin (HRP-SA)	10ml/20ml	2-8°C(Avoid Direct Light)
Biotin System (BS)	60ul/120ul	2-8°C
BS Dilution Buffer	5ml/10ml	2-8°C
TMB Substrate	5ml/10ml	2-8°C(Avoid Direct Light)
Stop Solution	5ml/10ml	2-8°C
Wash Buffer(25X)	30ml/30ml×2	2-8°C
Plate Sealer	3/5pieces	
Product Description	1copy	

### Typical Data & Standard Curve

Results of a typical standard operation of a IL-6 ELISA Kit are listed below. This standard curve was generated at our lab for demonstration purpose only. Users shall obtain standard curve as per experiment by themselves. (N/A=not applicable)

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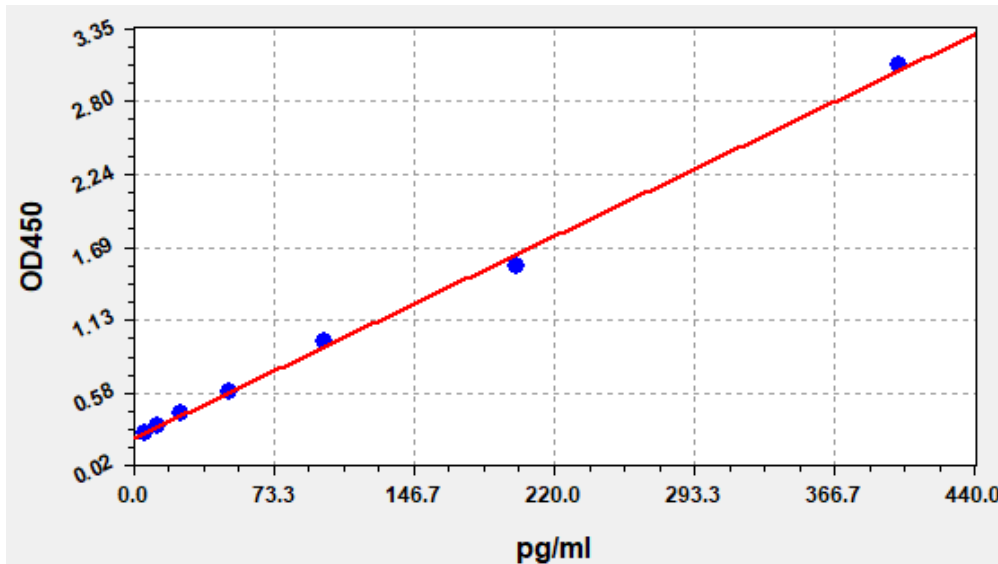
1st Floor, Building A02, Optics Valley Biomedical Industrial Park, Wuhan, China (430075)

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STD.(pg/ml)	OD-1	OD-2	Average	Corrected
0	0.217	0.237	0.227	0
6.25	0.282	0.271	0.277	0.05
12.5	0.341	0.327	0.334	0.107
25	0.434	0.417	0.426	0.199
50	0.586	0.601	0.594	0.367
100	0.954	0.977	0.966	0.739
200	1.521	1.558	1.540	1.313
400	3.032	3.106	3.069	2.842



### Specificity

This assay has high sensitivity and excellent specificity for detection of IL-6. No significant cross-reactivity or interference between IL-6 and analogues were observed.

Note: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between IL-6 and all the analogues, therefore, cross reaction may still exist.

### Recovery

Matrices listed below were spiked with certain level of IL-6 and the recovery rates were calculated by comparing the measured value to the expected amount of IL-6 in sample.

Matrix	Recovery Range (%)	Average (%)

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Serum(n=5)	83-97	94
EDTA Plasma(n=5)	88-104	98
Heparin Plasma(n=5)	87-104	95

### Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of IL-6 and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8
Serum(n=5)	85-102%	85-101%	91-103%
EDTA Plasma(n=5)	87-96%	91-98%	85-100%
Heparin Plasma(n=5)	85-102%	85-101%	91-103%

### Sample values

Cell Culture Supernates - Mouse peripheral blood mononuclear cells ( $1 \times 10^6$  cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 50  $\mu$ M  $\beta$ -mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 $\mu$ g/mL streptomycin sulfate and stimulated for 24h with 10 $\mu$ g/mL PHA. Aliquots of the culture supernates were removed and assayed for levels of mouse IL-6.

Condition	Con.(pg/ml)
Treated	3500
untreated	6.2

### Precision

Intra-Assay: CV<8%

Inter-Assay: CV<10%

### Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage condition.

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

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To minimize extra influence on performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is strongly suggested that the same operator performs the whole assay from the beginning to the end.

## Operation Procedure

### Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Please do not mix the reagents in different kits of our company. Do not mix reagents from other manufacturers.
10. To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.

### Material Required but Not Supplied

1. Microplate reader (wavelength:450nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

### Washing

**Manual:** Discard the solution in the plate without touching the side walls. Clap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350ul wash buffer and soak for 1 to 2 minutes, then aspirate contents from the plate, and clap the plate on absorbent filter papers or other absorbent material.

**Automatic:** Aspirate all wells, and then wash plate with 350ul wash buffer. After the final wash, invert plate, and clap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer shall be set for soaking 1 minute. (**Note:** set the height of the needles; be sure the fluid can be sipped up completely)

## [Sample Collection and Storage \(universal\)](#)

<https://www.fn-test.com/download/>



Please refer to the following link or qr code for detailed sample preparation guidelines

- **Serum:** Place whole blood sample at room temperature for 2 hours or put it at 2-8°C overnight and centrifugation for 20 minutes at approximately 1000×g, Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.
- **Plasma:** Collect plasma using EDTA-Na<sub>2</sub> or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.
- **Tissue Homogenates:** As hemolysis blood has relation to assay result, it is necessary to remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Mince tissue after weighing it and get it homogenized in PBS (the volume depends on the weight of the tissue. Normal, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000×g to get the supernatant. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3mg.
- **Adherent and Suspension Cell Culture:** Use three T25 flasks or one T75 flask for cell culture, the number of cells (1x10<sup>7</sup>);
  1. **Suspension cell:** centrifuge at 2500 rpm at 2-8°C for 5 minutes; collect clarified cell culture supernatant;
  2. **Adherent cell:** collect supernatant directly; centrifuge at 2500 rpm at 2-8°C for 5 minutes; collect clarified cell culture supernatant for immediate detection or store it separately at -80°C.
- **Cell Lysate Preparation:** Two types of cell lysates are specified below.
  1. **Suspension Cell Lysate :** Centrifuge at 2500 rpm at 2-8°C for 5 minutes; Then add pre-cooling PBS into collected cell and gently mix. Recollect cell by repeating centrifugation. Add 0.5-1ml RIPA lysis buffer (NP-40 lysis buffer or Triton X-100 surfactant is not recommended due to the interfering with antigen-antibody reaction). Add suitable protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Lyse the cell on ice for 30min-1h. During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. Alternatively, cells are subject to fragmentation by ultrasonic cell disruptor (300W, 3~5 s/time, 30s intervals, four-five times) or ultrasonic generator (14 μ m for 30s ). 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 and stored at -80°C.
  2. **Adherent Cell Lysate:** Absorb supernatant and add pre-cooling PBS once. Then, add 0.5-1ml RIPA lysis buffer (NP-40 lysis buffer or Triton X-100 surfactant is not recommended due to the interfering with antigen-antibody reaction). Add the suitable protease inhibitor (e.g. PMSF, working concentration: 1mmol/L). Scrape adherent cell gently with a cell scraper. Add the cell suspension into centrifugal tube. Lyse the cell on ice for 30min-1h. During lysate process, use the tip for pipetting or intermittently shake the centrifugal tube to completely lyse the protein. Alternatively, cells are subject to fragmentation by ultrasonic generator (14 μ m for 30s ) or ultrasonic cell disruptor (300W, 3~5 s/time, 30s intervals, four-five times). At the end of lysate/ultrasonic disruption, centrifuge at 10000rpm at 2-8°C for 10 minutes. Then, the supernatant is added into EP tube and stored at -80°C.

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- **Other Biological Fluids:** Centrifuge samples for 20 minutes at 1000×g at 2-8°C. Collect supernatant and carry out the assay immediately.

**Note:** Samples to be used within 5 days can be stored at 2-8°C, besides that, samples must be stored at -20°C (assay ≤1 month) or -80°C (assay ≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles. The hemolytic samples are not suitable for this assay.

## Sample Dilution

The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be making in pre-experiment. If samples with very high concentrations, dilute samples with PBS first and then dilute the samples with Sample Dilution.

## Reagent Preparation and Storage

Bring all reagents and samples to room temperature for 20 minutes before use.

### 1, Wash Buffer:

If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.

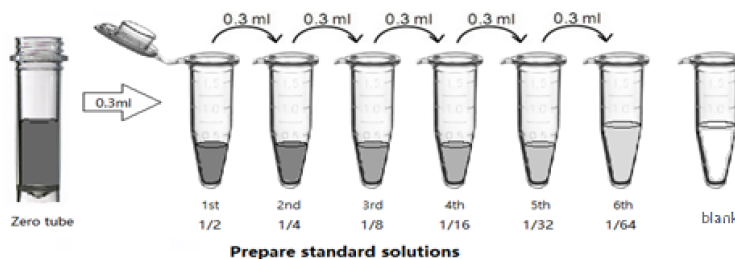
Dilute 30ml Concentrated Wash Buffer to 750ml Wash Buffer with deionized or distilled water. Put unused solution back at 2-8°C.

### 2, Standards:

1). Add 1 ml Sample Dilution Buffer into one Standard tube (labeled as zero tube), keep the tube at room temperature for 10 minutes and mix them thoroughly.

**Note: If the standard tube concentration higher than the range of the kit , please dilute it and labeled as zero tube.**

2). 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 of the above Standard solution (from zero tube) into 1st tube and mix them thoroughly. Transfer 0.3ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3ml from 2nd tube to 3rd tube and mix them thoroughly, and so on. Sample Dilution Buffer was used for the blank control.



**Note:** It is best to use Standard Solutions within 2 hours.

### 3, Preparation of BS Working Solution:

Prepare it within 15 minutes before experiment.

- 1 ) Calculate required total volume of the working solution: 0.1ml/well × quantity of wells. (Allow 0.1-0.2ml more than the total volume.)

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- 2 ) Dilute the Biotin System (BS) with BS Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1ul BS into 99ul BS Dilution Buffer.)

**NOT:** If crystals have formed in the BS, you can warm it with water (temperature should not exceed 30°C) and mix it gently until the crystals have completely been dissolved.

## Assay Procedure

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C. It is recommended to plot a standard curve for each test.

1. Set standard, test samples, control (blank) wells on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate.
2. Add 50µL of Assay Diluent to each well.
3. **Prepare Standards:** Aliquot 50ul of zero tube, 1<sup>st</sup> tube, 2<sup>nd</sup> tube, 3<sup>rd</sup> tube, 4<sup>th</sup> tube, 5<sup>th</sup> tube, 6<sup>th</sup> tube and Sample Dilution Buffer (blank) into the standard wells.
4. **Add Samples:** Add 50ul of properly diluted sample into test sample wells , mix by gently tapping the plate frame for 1 minute.
5. **Incubate:** Seal the plate with a cover and incubate at 37°C for 90 minutes.
6. **Wash:** Remove the cover and discard the plate content, and wash plate 2 times with Wash Buffer. Do NOT let the wells dry completely at any time.
7. **Biotin-labeled Antibody:** Add 100ul Biotin-labeled antibody into above wells (standard, test sample and blank wells). Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37°C for 60 minutes.
8. **Wash:** Remove the cover, and wash plate 2 times with Wash Buffer, and let the Wash Buffer stay in the wells for 1-2 minutes each time.
9. **HRP-Streptavidin (HRP-SA):** Add 100ul of HRP-SA into each well, cover the plate and incubate at 37°C for 30 minutes.
10. **Wash:** Remove the cover and wash plate 3 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minutes each time.
11. **BS Working Solution:** Add 100ul of BS Working Solution into each well, cover the plate and incubate at room temperature for 15 minutes
12. **Wash:** Remove the cover and discard the plate content, and wash plate 3 times with Wash Buffer.
13. **HRP-Streptavidin (HRP-SA):** Add 100ul of HRP-SA into each well, cover the plate and incubate at 37°C for 30 minutes.
14. **Wash:** Remove the cover and wash plate 3 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minutes each time.
15. **TMB Substrate Working Solution:** Add 90ul TMB Substrate Working Solution into each well, cover the plate and incubate at 37°C in dark within 10-20 minutes. (**Note:** 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.)
16. **Stop:** Add 50ul Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.
17. **OD Measurement:** Read the O.D. absorbance at 450nm in Microplate Reader immediately after adding the stop solution. Regarding calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X).

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The target concentration of the samples can be interpolated from the standard curve. It is recommended to use some professional software to do this calculation, such as **Curve Expert 1.3 or 1.4.**

**Note:** If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

## **Summary**

**Step1:** Add 50µL of Assay Diluent to each well.

**Step2:** Add 50ul standard or sample to each well and incubate for 90 minutes at 37°C.

**Wash step:** Aspirate and wash plates 2 times.

**Step3:** Add 100ul Biotin-labeled antibody to each well and incubate for 60 minutes at 37°C.

**Wash step:** Aspirate and wash plates 2 times.

**Step4:** Add 100ul HRP-SA into each well and incubate for 30 minutes at 37°C.

**Wash step:** Aspirate and wash plates 3 times.

**Step5:** Add 100ul BS Working Solution Incubate 15minutes at room temperature.

**Wash step:** Aspirate and wash plates 3 times.

**Step6:** Add 100ul HRP-SA into each well and incubate for 30 minutes at 37°C.

**Wash step:** Aspirate and wash plates 3 times

**Step7:** Add 90ul TMB Substrate Solution. Incubate 10-20 minutes at 37°C.

**Step8:** Add 50ul Stop Solution. Read at 450nm immediately and calculation.