IMMUNOPORT

SKU IP400173

ELISA KIT FOR MOUSE IL6

Research Use Only
For *in vitro* applications - not for consumption

+1-(647)-3722995
INFO@IMMUNOPORT.COM

VERSION 1.0

INTENDED USE

The kit is a sandwich enzyme immunoassay for in vitro quantitative measurement of Interleukin 6 (IL6) in mouse serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids.

KIT CONTENTS & STORAGE

Pre-coated Microplate [12 X 8 Strips]
Standard [2 vials]
Standard Diluent [20mL]
Detection Reagent A and B [120µL each]
Assay Diluent A and B [12mL each]
30X Wash solution [20mL]
TMB Substrate [9mL]
Stop Solution [6mL]
Plate Sealing Membrane [4pcs]
User manual [1pc]

The Standard, Detection Reagent A, Detection Reagent B and the 96-well strip plate should be stored at -20°C while the others could be at 4°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

ELISA Reader, 37 °C Thermostat, micropipettes and tips, Eppendorf tubes, absorbent paper, autowasher, deionized or distilled water.

TEST PRINCIPLE

The microplate provided in this kit has been pre-coated with an antibody specific to IL6. Standards or samples are then added to the appropriate microplate wells with a biotin-conjugated antibody specific to IL6. Next, Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. After TMB substrate solution is added, only those wells that contain IL6, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color. The enzyme-substrate reaction is terminated by the addition of sulphuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 10nm. The concentration of IL6 in the samples is then determined by comparing the O.D. of the samples to the standard curve.

CHARACTERISTICS

Sample Type: Serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids.

Detection Range: 7.8-500pg/mL
Minimum Detection Dose: 3.0pg/mL
Intra-Assay Precision: CV<10%
Inter Assay Precision: CV<12%

Cross Reactivity: None observed with analogues
Expiry: As per kit label
Storage (Unopened kit): -20°C in shelf life, 4°C (for upto a month)

Once opened unused wells should be put into the sealed bag with a desiccant pack and may be stored for up to 1 month at 2-8°C.

LINEARITY

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of IL6 and their serial dilutions. The results were demonstrated by the percentage of calculated concentration to the expected.

Sample	1:2	1:4	1:8	1:16
Serum (n=5)	84-98%	97-105%	81-95%	80-94%
EDTA plasma (n=5)	98-109%	89-103%	95-101%	97-99%
Heparin plasma (n=5)	83-92%	81-97%	82-96%	84-102%

RECOVERY

Matrices listed below were spiked with certain level of IL6 and the recovery rates were calculated by comparing the measured value to the expected amount of IL6 in samples.

Matrix	Recovery Range (%)	Average (%)
Serum (n=5)	80-95	87
EDTA plasma (n=5)	81-104	93
Heparin plasma (n=5)	83-97	92

SAMPLE COLLECTION

Serum - Use a serum separator tube and allow samples to clot for two hours at room temperature or overnight at 4°C before centrifugation for 20 minutes at approximately 1,000×g. Assay freshly prepared serum immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1,000×g at 2-8°C within 30 minutes of collection. Remove plasma and assay immediately or store samples in aliquot at -20°Cor -80°C for later use. Avoid repeated freeze/thaw cycles.

Tissue homogenates - Rinse the tissues with ice-cold PBS (0.01M, pH=7.4) to remove excess blood thoroughly. Tissue pieces should be weighed and then minced to small pieces and homogenized in PBS with a glass homogenizer on ice. Different lysis buffer needs to be chosen based on subcellular location of the target protein. 1mL lysis buffer should be added in 9 mg tissue sample. Protease inhibitor is recommended to be added into the PBS. To further break the cells, sonication of the suspension may be done with an ultrasonic cell disrupter or they may be subjected to freeze-thaw cycles. The homogenates are centrifugated for 5 minutes at 10,000×g to collect supernatant.

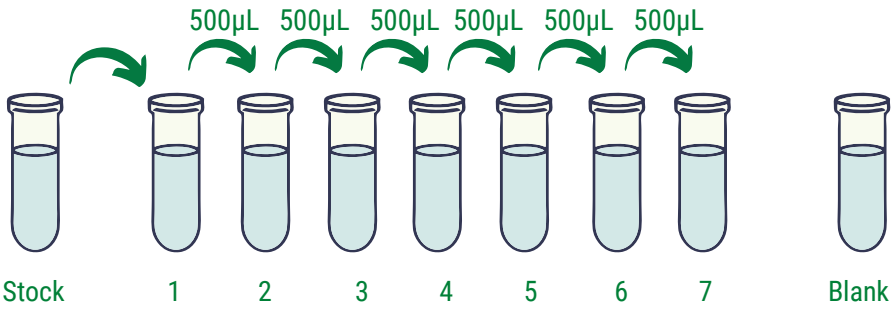
Cell Lysates - Cells need to be lysed before assaying according to the following directions. 1. Adherent cells should be washed by cold PBS gently, and then detached with trypsin, and collected by centrifugation at 1,000×g for 5 minutes (suspension cells can be collected by centrifugation directly). 2. Wash cells three times in cold PBS. 3. Resuspend cells in fresh lysis buffer with concentration of 107 cells/mL. If it is necessary, the cells could be subjected to ultrasonication till the solution is clarified. 4. Centrifuge at 1,500×g for 10 minutes at 2-8°C to remove cellular debris. Assay immediately or aliquot and store at ≤-20°C.

Cell culture supernates and other biological fluids - Centrifuge samples for 20 minutes at 1,000×g. Collect the supernates and assay immediately or store samples in aliquot at -20°C or -80°C for later use. Avoid repeated freeze/thaw cycles.
Note: Samples should be centrifuged adequately and no hemolysis or granule is to be allowed.

REAGENT PREPARATION

1. Bring all kit components and samples to room temperature before use. If the kit will not be used up in one time, please only take out strips and reagents for present experiment, and leave the remaining strips and reagents in required condition.

2. **Standard** - Reconstitute the Standard with 1.0mL of Standard Diluent, kept for 10 minutes at room temperature, shake gently(not to foam). The concentration of the standard in the stock solution is 2,000pg/mL. Please firstly dilute the stock solution to 500pg/mL and the diluted standard serves as the highest standard (500pg/mL). Then prepare 7 tubes containing 0.5mL Standard Diluent and use the diluted standard to produce a double dilution series according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 7 points of diluted standard such as500pg/mL, 250pg/mL, 125pg/mL, 62.5pg/mL, 31.2pg/mL, 15.6pg/mL, 7.8pg/mL, and the last EP tubes with Standard Diluent is the blank as 0pg/mL.



Concentrations

Stock- 2,000pg/mL
1- 500pg/mL
2- 250pg/mL
3- 125pg/mL
4- 62.5pg/mL
5- 31.2pg/mL
6- 15.6pg/mL
7- 7.8pg/mL
Blank- 0pg/mL

3. **Detection Reagent A and Detection Reagent B** - Briefly spin or centrifuge the stock Detection A and Detection B before use. Dilute them to the working concentration 100- fold with Assay Diluent A and B, respectively.

4. **Wash Solution** - Dilute 20mL of Wash Solution concentrate (30×) with 580mL of deionized or distilled water to prepare 600mL of Wash Solution (1×).

5. **TMB substrate** - Aspirate the needed dosage of the solution with sterilized tips and do not dump the residual solution into the vial again.

NOTE

1. Making serial dilution in the wells directly is not permitted.

2. Prepare standard within 15 minutes before assay. Please do not dissolve the reagents at 37°C directly.

3. Detection Reagent A and B are sticky solutions, therefore, slowly pipette them to reduce the volume errors.

4. Please carefully reconstitute Standards or working Detection Reagent A and B according to the instruction, and avoid foaming and mix gently until the crystals are completely dissolved. To minimize imprecision caused by pipetting, use small volumes and ensure that pipettors are calibrated. It is recommended to suck more than 10μL for one pipetting.

5. The reconstituted Standards, Detection Reagent A and Detection Reagent B can be **used only once**.

6. If crystals have formed in the Wash Solution concentrate (30×), warm to room temperature and mix gently until the crystals are completely dissolved.

SAMPLE PREPARATION

1. We are only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.

2.Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments. Sample should be diluted by PBS.

3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.

4. Tissue or cell extraction samples prepared by chemical lysis buffer may cause unexpected ELISA results due to the impacts from certain chemicals.

5. Due to the possibility of mismatching between antigen from other origin and antibody used in our kits (e.g., antibody targets conformational epitope rather than linear epitope), some native or recombinant proteins from other manufacturers may not be recognized by our products.

6. Influenced by the factors including cell viability, cell number or sampling time, samples from cell culture supernates may not be detected by the kit.

7. Fresh samples without long time storage is recommended for the test. Otherwise, protein degradation and denaturalization may occur in those samples and finally lead to wrong results.

PROCEDURE

1.Determine wells for diluted standard, blank and sample. Prepare 7 wells for standard, 1 well for blank. Add 100μL each of dilutions of standard (read Reagent Preparation), blank and samples into the appropriate wells. Cover with the Plate sealer. Incubate for 1 hour at 37°C.

2.Remove the liquid of each well, don't wash.

3.Add 100μL of Detection Reagent A working solution to each well, cover the wells with the plate sealer and incubate for 1 hour at 37°C.

4.Aspirate the solution and wash with 350μL of 1× Wash Solution to each well using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher, and let it sit for 1~2 minutes. Remove the remaining liquid from all wells completely by snapping the plate onto absorbent paper. Totally wash 3 times. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against absorbent paper.

5.Add 100μL of Detection Reagent B working solution to each well, cover the wells with the plate sealer and incubate for 30 minutes at 37°C.

6.Repeat the aspiration/wash process for total 5 times as conducted in step 4.

7.Add 90μL of Substrate Solution to each well. Cover with a new Plate sealer. Incubate for 10 - 20 minutes at 37°C (Don't exceed 30 minutes). Protect from light. The liquid will turn blue by the addition of Substrate Solution.

8.Add 50μLof Stop Solution to each well. The liquid will turn yellow by the addition of Stop solution. Mix the liquid by tapping the side of the plate. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

9.Remove any drop of water and fingerprint on the bottom of the plate and confirm there is no bubble on the surface of the liquid. Then, run the microplate reader and conduct measurement at 450nm immediately.

NOTE

1. **Assay preparation**: Keep appropriate numbers of wells for each experiment and remove extra wells from microplate. Rest wells should be resealed and stored at -20°C.

2. **Samples or reagents addition**: Please use the freshly prepared Standard. Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 minutes. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of standards, samples, and reagents. Also, use separated reservoirs for each reagent.

3. **Incubation**: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents are added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be controlled.

4. **Washing**: The wash procedure is critical. Complete removal of liquid at each step is essential for good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and false elevated absorbance reading.

5. **Controlling of reaction time**: Observe the change of color after adding TMB Substrate (e.g. observation once every 10 minutes), if the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.

6. TMB Substrate is easily contaminated. Please protect it from light.

7. The environment humidity which is less than 60% might have some effects on the final performance, therefore, a humidifier is recommended to be used at that condition.

CALCULATION

Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Create a standard curve with IL6 concentration on the y-axis and absorbance on the x-axis.
Draw a best fit curve through the points and it can be determined by regression analysis. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TROUBLESHOOTING

We understand research is challenging as it is but it can get very frustrating when experiments themselves don't go as they should. That is the precise reason why we strive to ensure that all our products work for the application shown in their respective data sheets.

We have compiled a list of most common errors and resolutions on our technical support webpage www.immunoport.com/scientific-support. Still if you have any concerns please feel free to write to us at info@immunoport.com

PRECAUTIONS

- Do not mix or substitute reagents with those from other lots or sources.
- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- Serum and plasma should be handled as potentially hazardous and capable of transmitting disease. Disposable gloves must be worn during the assay procedure, since no known test method can offer complete assurance that products derived from blood will not transmit infectious agents. Therefore, all blood derivatives should be considered potentially infectious and good laboratory practices should be followed.
- Any variation in standard diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Add sodium hypochlorite to a final concentration of 1.0% in the liquid waste generated. The waste should be allowed to stand for a minimum of 30 minutes to inactivate any viruses before disposal.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the ELISA Immunoassay, the possibility of interference cannot be excluded.

IMMUNOPORT

SCIENCE.SPEED.SMILE

FOR ORDERS / SUPPORT:

INFO@IMMUNOPORT.COM
+1-(647)-3722995