SARD BIOSCIENCES

Version: 1.01

Mouse Interleukin 6 (IL-6) ELISA Kit

Cat No: SB-EKM1086

Pack Size: 96Tests

FOR RESEARCH USE ONLY

NOT FOR INVITRO CLINICAL DIAGNOSTICS

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Intended Use

The ELISA kit is a Sandwich enzyme immunoassay technique for the in vitro quantitative determination of Mouse IL-6 concentrations in serum, plasma, tissue homogenates, cell lysates, cell culture supernates and other biological fluids.

Specification

1. Sensitivity:3pg/mL.
2. Detection Range:7.5-120pg/mL.
3. No significant cross-reactivity or interference between Mouse IL-6 and analogues was observed.
4. Repeatability: Coefficient of variation is < 13%.

Principle of the Procedure

This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Mouse IL-6. Standards or samples are added to the plate wells and combined with the specific antibody. Then Horseradish Peroxidase (HRP) labeled detection antibody specific for Mouse IL-6 is added to each micro plate well and incubated. And then, TMB substrate solution is added to all wells and incubated. An enzyme-catalyzed reaction generates a blue color in the solution, thereafter, stop solution is added to stop the substrate reaction and the color turns yellow. The yellow solution is read at a wavelength of 450nm. The concentration of Mouse IL-6 in the samples is then calculated from the OD value by establishing a standard curve.

Reagents & Materials Provided

Components	Specification	Storage
Pre-coated ELISA Plate	12wells ×8 strips	2-8℃
Standard	1vial: 0.5mL(240pg/mL)	2-8℃
HRP-Conjugate reagent	1×6mL	2-8℃
Standard Diluent	1×1.5mL	2-8℃
Sample Diluent	1×6mL	2-8℃
Substrate Reagent A	1×6mL	2-8℃
Substrate Reagent B	1×6mL	2-8℃
Wash Buffer (30×)	1×20mL	2-8℃
Stop Solution	1×6mL	2-8℃
Plate Sealer	2 pieces	
Manual	1 copy	
Self-sealing bags	1 copy	

Materials & Equipment Required but Not Provided

1.Microplate reader with 450nm wavelength filter
2.Incubator capable of maintaining 37℃
3.Single or multi-channel pipettes with high precision
4.disposable pipette tips
5.EP tubes
6.Container for Wash Solution
7.Squirt bottle, manifold dispenser, or automated microplate washer
8.Deionized or distilled water
9.Absorbent paper for blotting the microplate

Sample Collection

- **Serum:** Allow blood samples to clot for 2 hours at room temperature or overnight at 2-8℃ before centrifugation for 15 min at 1000×g at 2~8℃. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use.
- **Plasma :** Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge samples for 15 min at 2000×g at 2-8℃ within 30 min of collection. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use
- **Tissue homogenates :** Tissues should be rinsed in cool PBS to remove excess blood thoroughly, weighed, minced into small pieces. then Tissue pieces homogenized in cool PBS (tissue weight (g): PBS (mL) volume=1:9) with a glass homogenizer on ice. The resulting suspension is sonicated with an ultrasonic cell disrupter till the solution is clarified. The homogenates are then centrifuged for 5 min at 10000×g. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use.
- **Cell lysates:** For adherent cells, gently wash the cells with moderate amount of cool PBS and detach the cells with trypsin. Collect cells by centrifugation for 5 min at 1000×g(suspension cells can be collected by centrifugation directly). Discard the supernate and wash cells 3 times with cool PBS. Re-suspend cells in cool PBS with concentration of 5×106 cells/mL. Repeat the freeze-thaw process several times until the cells are fully lysed. Centrifuge for 10min at 1500×g at 2-8℃. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use.
- **Cell culture supernatant and other biological fluids:** Centrifuge samples for 20 minutes at 1000× g at 2-8℃. Supernatants should be taken for assay testing immediately or stored at -20℃ or -80℃ for later use.

Note for sample

1. Samples should be used within 6 days when stored at 2-8℃, otherwise samples must be stored at -20℃ (≤1month) or -80℃ (≤2months). Avoid repeated freeze-thaw cycles.
2. Please predict the concentration before assaying. If the sample concentration is not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.
3. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
4. If a lysis buffer is used to prepare tissue homogenates or cell culture supernatant, there is a possibility to cause a deviation due to the introduced chemical substance.
5. Some recombinant protein may not be detected due to a mismatching with the coated antibody or detection antibody.
6. Please do not use hemolysis samples for ELISA as it will affect the test results.
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 result.

Reagent Preparation

1. Bring all components to room temperature (18~25℃) before use. Follow the Microplate reader manual for set-up and preheat it for 15 min before OD measurement.
2. **Wash Buffer**: Dilute 20mL of Concentrated Wash Buffer with 580 mL of deionized or distilled water to prepare 600 mL of Wash Buffer.

3. **Standard Working Solution** Standard stock solution is 240pg/mL. Then make serial dilutions as needed.
   Take 6 EP tubes, add 150uL of Standard Diluent to each tube. Pipette 150uL of the 240pg/mL stock solution to the first tube and mix up to produce a 120pg/mL working solution. Pipette 150uL of the solution from the former tube into the latter one according to the picture shown below. Mix each tube thoroughly before the next transfer. Set up 6 points of diluted standard such as 120pg/mL, 60pg/mL, 30pg/mL, 15pg/mL, 7.5pg/mL and the last EP tubes with Standard Diluent is the blank as 0pg/mL.

Assay Protocol

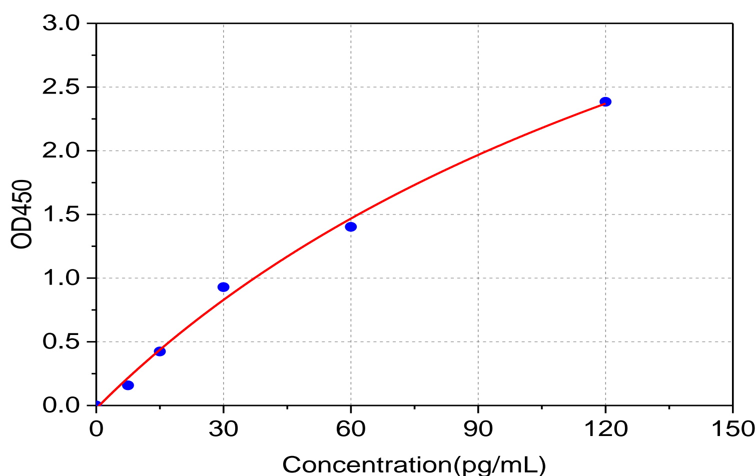
1 Determine wells for diluted standard, sample and blank separately (blank wells don't add sample and HRP-Conjugate reagent, other each step operation is same). Prepare 6 wells for standard points, add 50μL of standard solution to standard well, add 40μL of sample diluent to sample well, then add 10μL of sample, respectively. (sample final dilution is 5-fold)
2 Closing plate with plate Sealer, incubate for 30 min at 37℃.
3 Uncover plate Sealer, discard Liquid, add washing buffer to all wells, still for 30s then drain, repeat 5 times, dry by pat.
4 Add 50μL of HRP-Conjugate reagent to each well, except blank well, closing plate with plate Sealer, incubate for 30 min at 37℃.
5 Uncover plate Sealer, discard Liquid, add washing buffer to all wells, still for 30s then drain, repeat 5 times, dry by pat
6 Add 100μL of the equal volume mixture of Substrate Reagent A and Substrate Reagent B to each well, evade the light
7 preservation for 10min at 37℃. Add 50μL of Stop Solution to each well, Stop the reaction.
8 Read absorbance at 450nm within 15min.

Example data

The OD values of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), so plotting log of the data to establish a standard curve for each test is strongly recommended. Typical standard curve is provided below for reference only.

Calculation of Results

Average the duplicate readings for each standard and samples, then subtract the average zero standard optical density. Create a standard curve with standard concentration on the x-axis and OD values on the y-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.



Sensitivity

The minimum detectable dose of Mouse IL-6 is typically less than 3 pg/mL.

The sensitivity of this assay, or Limit of Detection (LOD) was defined as the lowest protein concentration that could be differentiated from zero. It was determined by adding three standard deviations to the mean OD value of twenty zero standard replicates and calculating the corresponding concentration.

Specificity

This assay has high sensitivity and excellent specificity for detection of Mouse IL-6 .

No significant cross-reactivity or interference between Mouse IL-6 and analogues was observed.

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

Precision

Intra-assay Precision (Precision within an assay): 3 samples with low, middle and high level Mouse IL-6 were tested on one plate, 8 replicates in the plate, respectively.

Inter-assay Precision (Precision between assays): 3 samples with low, middle and high level Mouse IL-6 were tested on 3 different plates, 8 replicates in each plate.

CV(%) = SD/mean × 100%

Intra-Assay: CV< 9%

Inter-Assay: CV< 13%

Recovery

Three matrices listed below were spiked with certain level of Mouse IL-6 , The recovery rates of Mouse IL-6 were calculated by comparing the measured value to the expected amount of Mouse IL-6 in samples.

Matrix type	Recovery Range (%)	Average (%)
Serum (n=5)	83-97	90
EDTA plasma (n=5)	86-97	92
Cell culture media	85-99	91

Linearity

Three types of Sample were spiked with appropriate concentrations of Mouse IL-6 and diluted into a series of concentration gradients, then the linearity of the assay was demonstrated by the percentage of comparing calculated concentrations and expected values.

Dilution Times	Recovery Range		
	Serum (n=5)	EDTA plasma (n=5)	Cell Culture Media (n=5)
1:2	96-104	85-98	96-109
1:4	88-95	86-95	85-99
1:8	91-104	88-98	104-115
1:16	91-104	89-105	96-109

Summary

Add 50μL standard or sample to each well. Incubate 30min at 37℃

Aspirate and wash 5 times

Add 50μL HRP-Conjugate reagent. Incubate 30 min at 37℃

Aspirate and wash 5 times

Add 100μL Substrate Reagent(A+B). Incubate 10 minutes at 37℃

Add 50μL Stop Solution

Read at 450nm within 15min

Calculation of results