Mouse IL-6 ELISA Kit

Product description

Interleukin-6 (IL-6) was initially identified as a B cell differentiation factor and is now known to be a multifunctional cytokine that regulates immune responses, hematopoiesis, acute-phase reactions, and inflammation. It has three receptor binding sites, including one specific receptor IL-6R binding site, and two gp130 binding sites. IL-6 is a pleiotropic, alpha-helical, 22~28 kDa glycoprotein that plays important roles in acute-phase reactions, inflammation, hematopoiesis, bone metabolism, and cancer progression. Human IL-6 shares 39% amino acid sequence identity with mouse and rat IL-6. It is a multifunctional cytokine that not only affects the immune system but also acts on various biological systems and physiological events in various organs, while also inducing the growth of bone marrow and plasma cell tumors.

The Arcegen Mouse IL-6 ELISA (Enzyme-Linked Immunosorbent Assay) Kit is an in vitro enzyme-linked immunosorbent assay kit used for the quantitative determination of mouse interleukin-6 (Mouse IL-6) in serum and plasma. Specific antibodies against mouse interleukin-6 are pre-coated on a high-affinity enzyme immunoassay plate. Standard samples and test samples are added to the wells of the plate, and after incubation, the mouse interleukin-6 present in the samples binds to the solid-phase antibody. After washing to remove unbound substances, a detection antibody is added and incubated for binding. After washing, enzyme conjugate (Streptavidin-HRP) is added and incubated for binding. After washing, a color substrate TMB is added for color development in the dark. The intensity of the color reaction is proportional to the concentration of mouse interleukin-6 in the sample. The reaction is terminated by adding a stop solution, and the absorbance is measured at 450 nm wavelength (with a reference wavelength of 570~630 nm).

Specifications

Catalog Number	P162003S/P162003E
Specifications	48 T/96 T
Detection Range	15.63~1000 pg/mL
Detection Method	ELISA
Species Detected	Mouse
Detection Time	4.5 hours
Sensitivity	9.37 pg/mL
Dilution Linearity	74~107%
Recovery	77~111%
Intra-assay Variation	3.1%

Inter-assay Variation	5.7%

Components

Component Number	Component Name	Storage Temperature	P162003S	P162003E
P162003-A	ELISA Plate	2~8°C	48 T	96 T
P162003-B	Standard	2~8°C	1 tube	2 tubes
P162003-C	Detection Antibody	2~8°C	120 μL	240 μL
P162003-D	Enzyme Conjugate	2~8°C(Avoid Light)	30 μL	60 μL
P162003-E	5×Dilution Buffer	2~8°C	8 mL	15 mL
P162003-F	20×Wash Buffer	2~8°C	25 mL	50 mL
P162003-G	Substrate Solution	2~8°C(Avoid Light)	8 mL	15 mL
P162003-H	Stop Solution	Room Temperature	5 mL	10 mL
P162003-I	Plate Sealant Film	Room Temperature	3 pieces	5 pieces

Shipping and Storage

Reagent Kit can be stored at 2~8°C or according to the storage conditions provided for each component to prevent contamination and repeated freeze-thaw cycles. Dilute reagents to working concentrations immediately before use and discard them afterward; they should not be reused. The shelf life is 1 year.

Table 1 Reagent Storage Table After Initial Use

Material Name	Storage Conditions
Enzyme Plate	Unused strips can be returned to aluminum foil pouch, tightly sealed, and stored at 2~8°C to avoid moisture absorption.
Standard	Use within 48 hours after dissolution, store at 2~8°C to avoid contamination.
Detection Antibody	Use within 48 hours after dilution, store at 2~8°C to avoid contamination.
Enzyme Conjugate	
5×Dilution Buffer	Store at 2~8°C for 1 month, avoid contamination
20×Wash Solution	
Substrate Solution	Store at 2~8°C for 1 month, protect from light.
Stop Solution	Can be stored at room temperature
Sealing Film	

Notes

1. For your safety and health, please wear lab coats and disposable gloves during operation.
2. The reagent kit should be used within its shelf life. Mixing different batches of related reagents

is prohibited.

3. This product is only intended for the detection of target antigens specified in the instruction manual and samples. Other applications must be designed and validated by the user, and the reliability and accuracy of the results should be assessed accordingly.
4. Do not mix or substitute reagents or materials from different batches of other reagent kits' suppliers.
5. For research use only.

Instructions

1. Used for quantitative detection of IL-6 content in serum, plasma, and cell culture supernatant.
2. Please read the instructions carefully before using this product.

Common Technical Tips

1. When the sample OD value is higher than the S1 OD value, further dilution should be performed in an appropriate diluent.
2. Avoid generating foam during mixing.
3. Replace pipette tips promptly when adding standards, samples, and others to avoid cross-contamination.
4. Ensure proper sealing of the microplate or complete coverage with sealing film during the incubation period.
5. Completely remove all solutions and buffer solutions during the cleaning steps.
6. Do not invert the standard solution tube before dissolving the standard. After inverting the standard solution tube, thoroughly mix it up and down after adding the buffer solution, then centrifuge at low speed.
7. Place reagents according to the instructions during the experiment.
8. Discard buffer solutions promptly after completing the experiment; use them once and then discard.
9. Reagent kit components vary among different products and should not be used interchangeably.

Other Preparation Materials

1. Plate reader, measuring absorbance at 450 nm (reference wavelength: 630 nm).
2. Incubator, automatic microplate washer.
3. Pipettes, ranging from 1 μL to 1 mL with corresponding tips.
4. Graduated cylinders, 100 mL and 1 L.
5. Tubes for diluting standards or samples.
6. Blotting paper.

7. Distilled water or deionized water.
8. Computer and analysis software.

Pre-Experiment Preparation

1. Sample Collection and Processing

1) Cell culture supernatant: Centrifuge at 1, 000×g for 10 minutes to remove precipitates, then immediately detect or aliquot and store at -20°C or below.

2) Serum samples: Collect serum using tubes free from pyrogens and endotoxins. After blood clotting for 30 minutes, centrifuge at 1, 000×g for 10 minutes. Immediately detect after aspirating serum samples, or aliquot and store at -20°C or below.

3) Plasma samples: Collect plasma samples using EDTA, sodium citrate, or heparin anticoagulants. Centrifuge at 1, 000×g for 30 minutes to collect samples. Immediately detect or aliquot and store at -20°C or below.

This assay kit may be applicable to other biological samples. Serum and cell culture supernatants have been validated.

【Note】Visible precipitates in samples must be removed before testing. Do not use samples with severe hemolysis or high lipids. Samples should be aliquoted and stored at -20°C to prevent loss of IL-6 activity. If testing within 24 hours, samples can be stored at 2~8°C, avoiding repeated freeze-thaw cycles. Before testing, frozen samples should be slowly equilibrated to room temperature (25°C±3°C) and gently mixed.

If sample dilution is necessary, use the specified sample diluent for dilution.

Recommended dilution for normal serum/plasma samples (for reference only): 1:1 dilution with 1× dilution buffer.

Recommended dilution for cell culture supernatants (for reference only): undiluted.

Due to variations in target protein content in samples, the dilution ratio for each sample should be determined based on preliminary experiment results or actual conditions.

2. Preparation of the ELISA Plate

The ELISA plate should be equilibrated to room temperature before use. Unused strips should be promptly sealed with desiccants and stored at 2~8°C. It is recommended to perform a multi-well assay for each sample.

3. Reagent Preparation

All reagent components and samples to be tested should be equilibrated to room temperature before use. To ensure experimental accuracy, please complete the preparation within 15 minutes before use.

1) Preparation of 1×Wash Buffer: Equilibrate the concentrated solution to room temperature

and dissolve completely without any crystallization. Mix well, take 25 mL of 20×Wash Buffer and dilute it with distilled water to 500 mL; adjust the volume according to the amount needed for each use.

2) Preparation of 1×Dilution Buffer: Equilibrate the concentrated solution to room temperature and dissolve completely without any crystallization. Mix well, take 10 mL of 5×Dilution Buffer and dilute it with distilled water to 50 mL; adjust the volume according to the amount needed for each use. The 1×Dilution Buffer is used to dilute standard solutions, samples to be tested, detection antibodies, and enzyme conjugates.

3) Preparation of Detection Antibodies: Before use, centrifuge at 10, 000 rpm for 20 seconds, then dilute with 1×Dilution Buffer at a 1:50 ratio for working concentration. For example, take 120 μL and dilute it with 1×Dilution Buffer to 6 mL; adjust the volume according to the amount needed for each use and mix well.

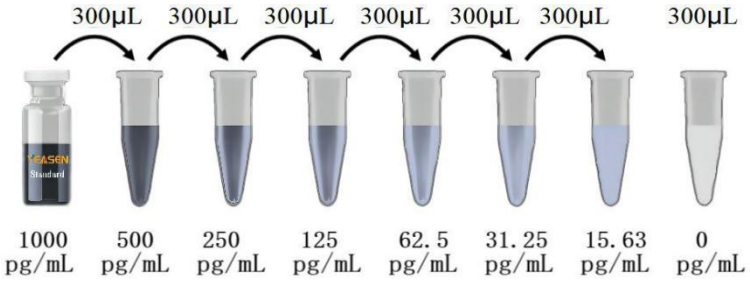
4) Preparation of Enzyme Conjugates: Before use, centrifuge at 10, 000 rpm for 20 seconds, then dilute with 1×Dilution Buffer at a 1:200 ratio for working concentration. For example, take 30 μL and dilute it with 1×Dilution Buffer to 6 mL; adjust the volume according to the amount needed for each use and mix well.

5) Preparation of Standard Curve: Prepare 7 sterile 1.5 mL centrifuge tubes and label them according to the standard concentrations. Prepare S1: Dissolve the lyophilized standard with distilled water according to the labeled amount, mix well to prepare a 1000 pg/mL solution. Add 300 μL of 1×Dilution Buffer to each centrifuge tube. Take 300 μL of S1 and mix well in the first centrifuge tube, then take 300 μL to the next labeled concentration tube and mix well to create a series of 2-fold diluted standard solutions. The starting highest concentration is labeled as 1000 pg/mL and the lowest concentration is 15.63 pg/mL, which can be prepared according to the following method. A corresponding standard curve should be prepared for each assay, and standard curves from different assay kits or different time points should not be mixed. When testing samples, the amount of standard solution required for each well is 100 μL. Ensure that the volume prepared is higher than the required volume to avoid insufficient usage.

Table 2 Preparation of IL-6 Standard Solutions (15.63~1000 pg/mL)

Standard Curve	Diluent (μL)	Volume of Standard Added (μL)	Final Concentration of Standard (ng/mL)
S1	As labeled	/	1000
S2	300	300	500
S3	300	300	250
S4	300	300	125
S5	300	300	62.5
S6	300	300	31.25
S7	300	300	15.63

Blank	300	0	0



Operating Instructions

Before use, all reagents and samples need to be equilibrated to room temperature. It is strongly recommended to perform duplicate measurements for all standards and test samples.

1. Reagent Preparation: Prepare various test reagents, dilute standard solutions, and test samples.
2. Determination of enzyme-labeled plate strips: Calculate the number of enzyme-labeled plate strips required for test samples and standard solutions. Remove the enzyme-labeled plate strips from the aluminum foil bag, return the remaining strips to the bag, seal the bag tightly, and store at low temperature.
3. Soak enzyme-labeled plate: Add 1×washing solution (350 μL/well) to soak the enzyme-labeled plate. After standing for 30 seconds, discard the liquid from the wells and tap the enzyme-labeled plate dry. The liquid volume has a significant impact on the test results, ensure that there is no residual washing solution after the final tapping.
4. Sample incubation: Add various gradient standard solutions and diluted test samples, 100 μL/well, ensuring that the spot sampling is completed within 15 minutes. Incubate at room temperature for 2 hours.
5. Washing the enzyme-labeled plate: Discard the liquid from the wells, add 1×washing solution (350 μL/well), wash the plate 5 times, and tap the enzyme-labeled plate dry.
6. Detection antibody incubation: Add the detection antibody pre-diluted to the working concentration into the enzyme-labeled plate, 100 μL/well, and incubate at room temperature for 2 hours.
7. Washing the enzyme-labeled plate: Discard the liquid from the wells, add 1×washing solution (350 μL/well), wash the plate 5 times, and tap the enzyme-labeled plate dry.
8. Enzyme binding incubation: Add the enzyme binding solution pre-diluted to the working concentration into the enzyme-labeled plate, 100 μL/well, and incubate at room temperature for 20 minutes.
9. Washing the enzyme-labeled plate: Discard the liquid from the wells, add 1×washing solution (350 μL/well), wash the plate 5 times, and tap the enzyme-labeled plate dry.

10. Color development: Before use, equilibrate the substrate solution to room temperature for 10 minutes. Add the substrate solution to the enzyme-labeled plate, 100 μL/well, and incubate at room temperature in the dark for 15 minutes.
11. Termination: Add 50 μL/well of stop solution to the enzyme-labeled plate. At this point, the color changes from blue to yellow. Gently shake the enzyme-labeled plate to ensure uniform color development.
12. Reading: Read the absorbance values at 450 nm/630 nm within 10 minutes.

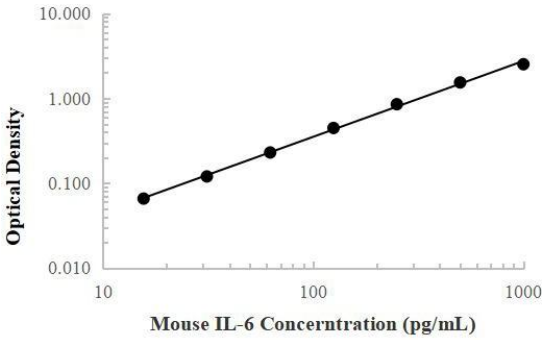
Standard Curve Establishment

Calculate the average OD values of duplicate wells for both standard solutions and samples, and subtract the average OD value of blank wells to obtain the calibrated OD value. Plot the standard curve with the logarithm of the concentration of standard solutions on the x-axis and the logarithm of the calibrated OD values on the y-axis. Various plotting and statistical software can be used to assist in drawing the standard curve and calculating the concentration of unknown samples. The four-parameter fitting method often yields better fitting results, while other methods such as linear fitting may also yield good fitting results, depending on the specific experimental data for analysis.

Experimental Data

1. Standard Curve Data

Data were fitted to generate a standard curve graph, which was used for the analysis of experimental data.



Standard Curve Graph

Concentration (ng/mL)	Absorbance		Mean Value	Calibration Value
1000	2.561	2.491	2.526	2.525
500	1.561	1.510	1.536	1.540
250	0.886	0.841	0.864	0.854
125	0.423	0.454	0.438	0.448

62.5	0.229	0.228	0.228	0.231
31.25	0.126	0.125	0.126	0.120
15.63	0.070	0.067	0.069	0.066
0	0.012	0.012	0.012	/

2. Sensitivity Detection

The minimum detection limit of IL-6 is 9.37 pg/mL, calculated by using the mean and standard deviation of zero-hole OD values repeated 20 times.

3. Precision Detection

Intra-assay Precision:

Three samples of known concentrations were assayed eight times to evaluate the precision within the ELISA plate.

Inter-assay Precision:

Three samples of known concentrations were assayed 24 times to evaluate the precision between ELISA plates.

Project	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
	8	8	8	24	24	24
Mean Value	614.01	165.44	39.27	622.44	161.44	42.01
Standard Deviation	16.25	5.85	1.29	24.60	6.54	3.82
Coefficient of Variation (%)	2.6	3.5	3.3	4.0	4.0	9.1

4. Recovery Rate Detection

The recovery rate was determined by adding IL-6 at different levels to the samples. The recovery rates are as follows:

Sample Types	Average Recovery Rate (%)	Range (%)
Serum	94.4	85.5~108.6
Plasma	96.3	81.6~111.1
Cell culture supernatant	90.2	77.5~102.2

5. Dilution Linearity Test

Serum Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	81.6	77.8~87.1
1:04	94.4	91.3~98.9
1:08	96.8	92.7~103.9
1:16	97.6	88.5~103.6

Plasma Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	94.5	84.0~107.2
1:04	96.2	88.9~100.2
1:08	102.3	99.0~106.7
1:16	82.2	74.4~92.5

Cell Culture Supernatant Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	86.3	82.5~89.6
1:04	95.4	93.3~98.1
1:08	97.7	91.7~102.7
1:16	97.2	96.0~99.4

6. Sample Values

Using this assay kit, samples from several healthy volunteers were tested. The medication history of the volunteers is unknown.

Sample Types	Sample Number	Mean Value (ng/mL)	Sample Value (ng/mL)
Serum	10	n. d.	n. d.
Plasma	8	33.6	n. d. ~59.0
Cell Culture Supernatant	4	n. d.	n. d.

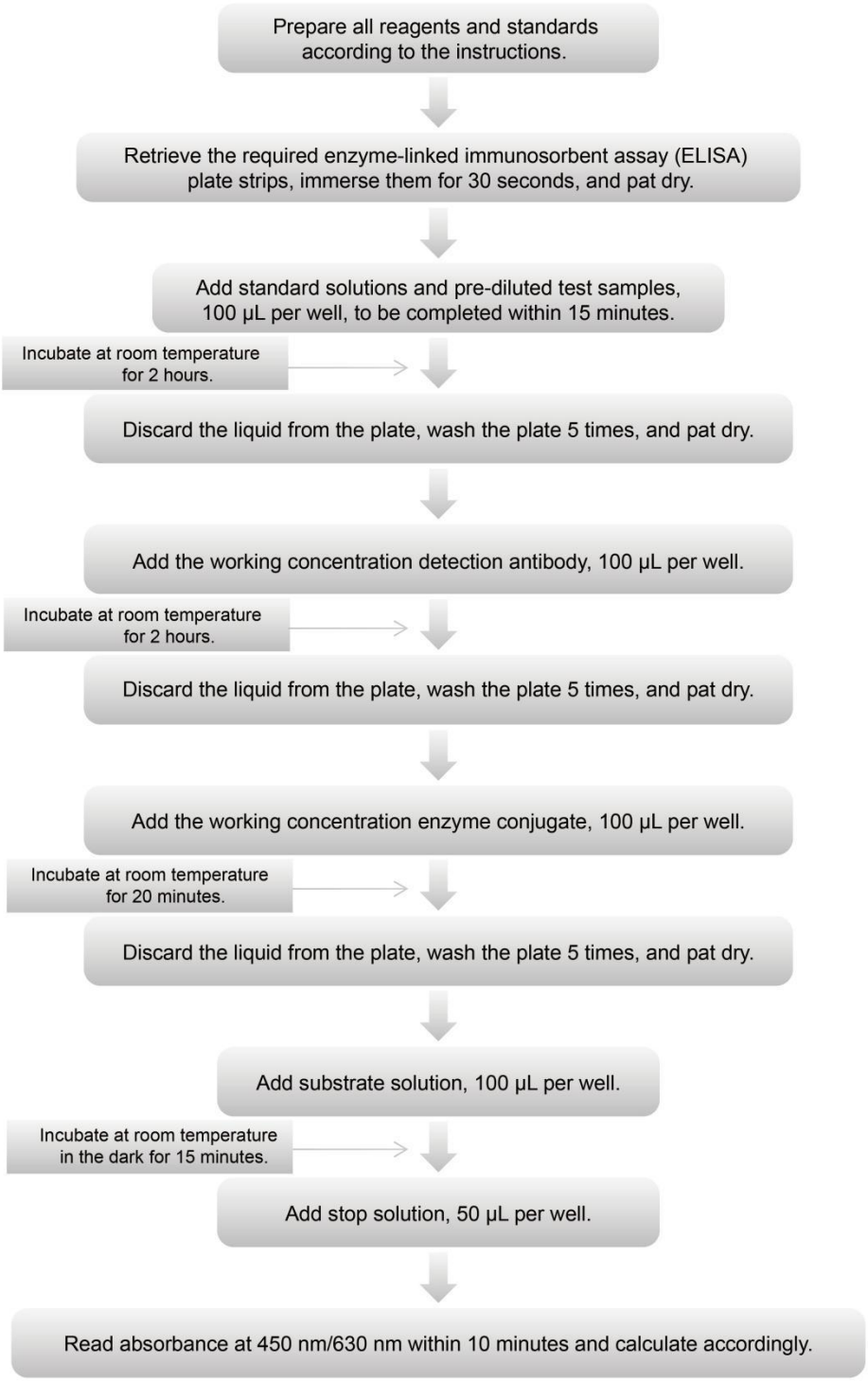
n. d. indicates that the sample concentration is below the detection range of 15. 63 pg/mL.

7. Specificity Assay

This assay kit identifies natural and recombinant mouse interleukin-6. Specificity was assessed using the following factors, and no significant cross-reactivity or interference effects were observed.

Recombinant human:		Recombinant Mouse:	
IL-1α	IL-8	IL-1α	IL-10
IL-1β	IL-10	IL-1β	IL-12
IL-2	IL-12	IL-2	IFN-γ
IL-4	IFN-γ	IL-4	VEGF
IL-5	VEGF	IL-5	TNF-α
IL-6	TNF-α		

Detection Schematic



Frequently Asked Questions

Issues	Causes	Solution
Poor standard curve	Inaccurate pipetting volumes	Check the pipette, calibrate it on time, handle it carefully, and tightly cover the pipette tip while avoiding foam as much as possible.
	Improper dilution method	
Low color intensity	Insufficient incubation time	Provide sufficient incubation time and replace the samples and dissolved standard overnight.
	Inadequate pipetting volume or improper dilution	Calibrate the pipette and adhere to standardized procedures.
High coefficient of variation (CV)	Improper washing of the ELISA plate	Utilize the correct washing procedure;if using a plate washer, check all ports for blockages.
	Contaminated wash buffer	Prepare fresh wash buffer.
Low sensitivity	Improper storage of the assay kit	Store according to the product component table.