Human IL-6 ELISA Kit

Product description

Interleukin-6 (IL-6) was initially identified as a B cell differentiation factor. It 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 binding site for IL-6R and two binding sites for gp130. IL-6 is a multifunctional, α-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 pleiotropic cytokine that not only affects the immune system but also acts on various physiological events in other biological systems and organs. It can also induce the growth of myeloma and plasma cell tumors.

The Arcegen Human IL-6 ELISA (Enzyme-Linked Immunosorbent Assay) Kit is an in vitro enzyme-linked immunosorbent assay kit used for quantitatively measuring Human Interleukin 6 (Human IL-6) in serum and plasma. Specific anti-Human Interleukin 6 antibodies are precoated onto high-affinity enzyme plates. Standard samples and test samples are added to the enzyme plate wells and incubated. Human Interleukin 6 in the samples binds to the solid-phase antibodies. After washing away unbound substances, detection antibodies are added and incubated, followed by washing and addition of enzyme conjugate (Streptavidin-HRP) for incubation. After washing, TMB substrate solution is added for color development in the dark. The intensity of the color reaction is directly proportional to the concentration of Human Interleukin 6 in the sample. The reaction is terminated by adding a stop solution, and the absorbance is measured at a wavelength of 450 nm (with a reference wavelength of 570 - 630 nm).

Specifications

Item Number	P162011S / P162011E
Specification	48 T / 96 T
Detection Range	7.81-500 pg/mL
Detection Method	Sandwich ELISA
Species Detected	human
Detection Time	4.5 hours
Sensitivity	2.53 pg/mL
Dilution Linearity	75 - 116%
Recovery Rate	75 - 108%
Intra-assay Variability	6.1%
Inter-assay Variability	7.6%

Components

Component Number	Component Names	Storage Temperature	P162011S	P162011E
P162011-A	ELISA plate	2~8°C	48 T	96 T
P162011-B	Standard sample	2~8°C	1 tube	2 tubes
P162011-C	Detection antibody	2~8°C	120 μL	240 μL
P162011-D	Enzyme conjugate	2~8°C(Avoid Light)	30 μL	60 μL
P162011-E	5× dilution buffer	2~8°C	8 mL	15 mL
P162011-F	20× wash buffer	2~8°C	25 mL	50 mL
P162011-G	Substrate solution	2~8°C(Avoid Light)	8 mL	15 mL
P162011-H	Stop solution	Room Temperature	5 mL	10 mL
P162011-I	Plate sealing film	Room Temperature	3 pieces	5 pieces

Storage

The kit can be stored at 2~8°C, or according to the storage conditions of individual components to avoid contamination and repeated freeze-thaw cycles. Diluted reagents prepared to working concentration should be used immediately and discarded; they should not be reused. The shelf life is 1 year.

Table 1. Reagent Storage Table After Initial Use

Component Names	Storage Conditions
ELISA plate	Unused strips can be returned to the aluminum foil bag,tightly sealed,and stored at 2-8°C to avoid moisture absorption.
Standard sample	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,avoiding contamination
20× wash buffer	
Substrate solution	Store at 2~8°C for 1 month,avoiding light exposure.
Stop solution	Can be stored at room temperature
Plate sealing film	

Instructions

1. For quantitative determination of interleukin-6 (IL-6) content in serum, plasma, and cell culture supernatants.

2. Please read the instruction manual carefully before using this product.

Notes

1. This product is for research use only.
2. For your safety and health, please wear lab coat and disposable gloves while handling.
3. The kit should be used within the shelf life. Mixing reagents from different batches is prohibited.
4. This product is intended for detection of target antigens and samples as indicated in the instruction manual. Other applications must be validated and evaluated for reliability and accuracy by the user.
5. Do not mix or substitute reagents or materials from different kit batches or suppliers.

Common Technical Tips

1. If the sample OD value is higher than the S1 OD value, further dilution should be done in an appropriate diluent.
2. Avoid generating foam during mixing.
3. When adding standards, samples, and other reagents, replace pipette tips in a timely manner to prevent cross-contamination.
4. Ensure proper sealing of the microplate or cover the plate with sealing film during incubation.
5. Completely remove all solutions and buffers during the washing steps.
6. Do not invert the standard vial before dissolving. After inverting the standard vial, thoroughly mix it with the buffer by gentle agitation and then centrifuge at low speed.
7. Place reagents according to the instructions during the experiment.
8. Discard buffers promptly after completing the experiment; do not reuse.
9. Components of different product kits vary and should not be cross-used.

Other Preparation Materials

1. ELISA reader, measure absorbance at 450 nm (reference wavelength 630 nm).
2. Incubator, automated microplate washer.
3. Pipettes, 1 μL to 1 mL pipettes with corresponding tips.
4. 100 mL and 1 L graduated cylinders.
5. Standard or sample dilution tubes.
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. Test immediately or aliquot and store at -20°C or below.

2) Serum samples: Collect serum using tubes free from pyrogens and endotoxins. Allow blood samples to clot for 30 minutes, then centrifuge at 1, 000 × g for 10 minutes. Test immediately after collecting 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. Test immediately or aliquot and store at -20°C or below.

This assay kit may be suitable for other biological samples. Serum and cell culture supernatant have been validated.

[Note] Remove visible precipitates from samples before testing. Do not use samples with severe hemolysis or high lipids. Store samples in aliquots at -20°C to prevent loss of IL-6 activity. If testing within 24 hours, samples can be stored at 2~8 °C to avoid repeated freeze-thaw cycles. Before testing, slowly bring frozen samples to room temperature (25°C ± 3°C) and gently mix.

If samples need dilution, use the specified sample diluent for dilution.

Recommended dilution for cell culture supernatant (for reference only): Undiluted.

The dilution ratio for each sample should be determined based on pre-experimental results or actual conditions due to variations in the target protein content in samples.

2. Preparation of the ELISA Plate

The ELISA plate should be brought to room temperature before use.Unused strips should be promptly sealed in a desiccant and stored at 2~8°C, with each sample requiring multiple wells for experimentation.

3. Reagent Preparation

Before use, all reagents and samples need to be equilibrated to room temperature. To ensure the accuracy of the experiment, please complete the equilibration within 15 minutes before use.

1) 1 × Wash Buffer Preparation: Equilibrate the concentrated solution to room temperature, dissolve thoroughly without crystallization. Mix well, take 25 mL of 20 × Wash Buffer and dilute it with distilled water to a total volume of 500 mL; the specific volume can be prepared according to the amount needed for each use.

2) 1 × Dilution Buffer Preparation: Equilibrate the concentrated solution to room temperature, dissolve thoroughly without crystallization. Mix well, take 10 mL of 5 × Dilution Buffer and dilute it with distilled water to a total volume of 50 mL; the specific volume can be prepared according to

the amount needed for each use. 1 × Dilution Buffer is used to dilute standard samples, test samples, detection antibodies, and enzyme conjugates.

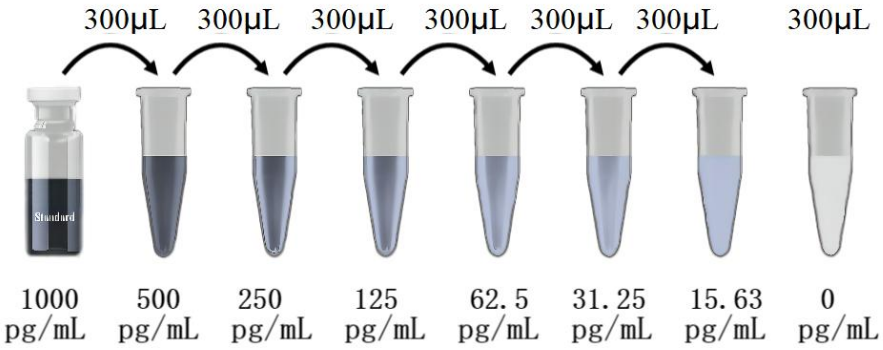
3) Detection Antibody Preparation: Before use, centrifuge at 10000 rpm for 20 seconds, then dilute with 1×Dilution Buffer at a ratio of 1:50 to the working concentration, for example: take 120 µL and dilute it to 6 mL with 1×Dilution Buffer; the specific volume can be prepared according to the amount needed for each use, mix thoroughly.

4) Enzyme Conjugate Preparation: Before use, centrifuge at 10000 rpm for 20 seconds, then dilute with 1×Dilution Buffer at a ratio of 1:200 to the working concentration, for example: take 30 µL and dilute it to 6 mL with 1 × Dilution Buffer; the specific volume can be prepared according to the amount needed for each use, mix thoroughly.

5) Preparation of Standard Curve: Prepare 7 sterile 1.5 mL centrifuge tubes and label them according to the standard concentration. S1 Preparation: Dissolve the specified amount of standard lyophilized product with distilled water according to the label, mix thoroughly, label it as 500 pg/mL. Add 300 µL of 1×Dilution Buffer to each centrifuge tube, first add 300 µL of S1 to the first tube, mix thoroughly, then add 300 µL to the next tube with the next labeled concentration, mix thoroughly, to prepare a series of 2-fold dilution standard samples, starting from the highest concentration labeled as 500 pg/mL, the lowest concentration is 7.81 pg/mL, can be prepared according to the following method. Prepare a corresponding standard curve for each experiment, standard curves from different assay kits or different times should not be mixed. For sample testing, 100 µL of standard is required per well, ensure that the volume prepared is higher than the required volume to avoid insufficient usage.

Table 2. IL-6 Standard Curve Preparation (7.81-500 pg/mL)

Standard Curve	Diluent(µL)	Volume of Standard Added(µL)	Final Concentration of Standard (pg/mL)
S1	As labeled	/	500
S2	300	300	250
S3	300	300	125
S4	300	300	62.5
S5	300	300	31.25
S6	300	300	15.63
S7	300	300	7.81
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 samples.

1. Reagent Preparation: Prepare all reagents needed for testing, including diluted standard solutions and samples.
2. Determine Number of Plates: Calculate the number of plates required for samples and standards. Remove the required number of plates from the aluminum foil bag, reseal the remaining plates, and store them at low temperature.
3. Plate Soaking: Add 1× wash buffer (350 μL/well) to soak the plates. After 30 seconds, discard the liquid from the wells and tap dry the plates. Ensure no residual wash buffer remains after the final tapping.
4. Sample Incubation: Add each gradient of standard solutions and pre-diluted samples, 100 μL/well. Ensure the completion of pipetting within 15 minutes and incubate at room temperature for 2 hours.
5. Plate Washing: Discard the liquid from the wells and wash the plates 5 times with 1× wash buffer (350 μL/well). Tap dry the plates.
6. Detection Antibody Incubation: Add the detection antibody pre-diluted to working concentration to the plates, 100 μL/well. Incubate at room temperature for 2 hours.
7. Plate Washing: Discard the liquid from the wells and wash the plates 5 times with 1× wash buffer (350 μL/well). Tap dry the plates.
8. Enzyme Conjugate Incubation: Add the enzyme conjugate pre-diluted to working concentration to the plates, 100 μL/well. Incubate at room temperature for 20 minutes.
9. Plate Washing: Discard the liquid from the wells and wash the plates 5 times with 1× wash buffer (350 μL/well). Tap dry the plates.
10. Color Development: Equilibrate the substrate solution to room temperature for 10 minutes. Add the substrate solution to the plates, 100 μL/well. Incubate at room temperature in the dark for 15 minutes.
11. Termination: Add 50 μL/well of stop solution to the plates. The color will change from blue to yellow. Gently shake the plates to ensure uniform color development.

12. Reading: Read the absorbance values at 450 nm/630 nm within 10 minutes.

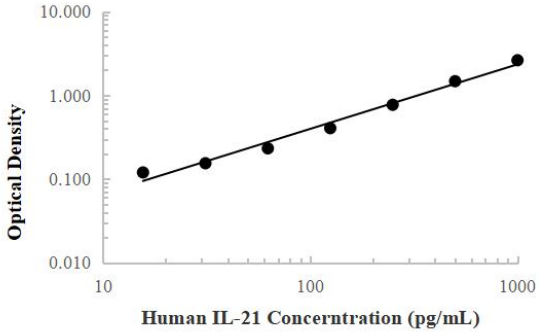
Standard Curve Establishment

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

Experimental Data

1. Standard Curve Data

Fitting data to generate a standard curve for analysis of experimental data.



Standard Curve Graph

Concentration(pg/mL)	Absorbance		Mean Value	Calibration Value
500	2.943	2.928	2.935	2.938
250	2.024	1.973	1.999	1.985
125	1.201	1.206	1.203	1.228
62.5	0.743	0.724	0.733	0.717
31.25	0.412	0.405	0.409	0.410
15.63	0.247	0.239	0.243	0.238
7.81	0.140	0.135	0.138	0.145
0	0.037	0.032	0.035	/

2. Sensitivity Detection

TThe minimum detection limit of IL-6 is 2.53 pg/mL, calculated by using the mean and standard deviation of zero well OD values from 20 repeated detections.

3. Precision Detection

Intra-assay Precision

The precision within the enzyme-linked immunosorbent assay (ELISA) plate was assessed by repeating the measurement of three samples with known concentrations ten times, evaluating the intra-plate precision.

Inter-assay Precision

The precision between ELISA plates was assessed by repeating the measurement of three samples with known concentrations thirty times, evaluating the inter-plate precision.

project	Intra-assay Precision			Inter-asaay Precison		
Sample	1	2	3	1	2	3
	10	10	10	30	30	30
Mean Value	199.7	46.3	14.7	203.9	46.7	14.5
Standard Deviation	15.0	1.6	1.1	17.7	2.3	1.3
Coefficient of Variation (%)	7.5	3.4	7.3	8.7	4.9	9.2

4. Recovery Rate Detection

Recovery rates were determined by adding IL-6 at different levels to the samples, and the recovery rates are as follows:

Sample Types	Average Recovery Rate (%)	Range (%)
Cell culture supernatant	94.5	83.2-108.8
Serum	84.4	75.4-94.4
Plasma	91.4	83.6-100

5. Dilution Linearity Test

Cell Culture Supernatant Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	95.9	92.4-101.5
1:04	92.6	89.2-97.8
1:08	96.1	91.8-104.6
1:16	85.4	79-88.5

Serum Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	93.1	85.5-99.6
1:04	99.7	86.3-107.1

1:08	98.5	93.1-104.2
1:16	88.7	80-98.6

Plasma Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	97.0	87.7-116.4
1:04	104.0	95.5-116.8
1:08	102.9	97.5-110.6
1:16	87.1	75.3-93.7

6. Sample Values

The assay kit was applied to detect samples from several healthy volunteers, whose medication history was unknown.

Sample Types	Sample Number	Mean Value (pg/mL)	Sample Value(pg/mL)
Cell Culture Supernatant	8	n.d.	n.d.
Serum	12	25.8	n.d.-263.4
Plasma	8	22.2	n.d.-177.58

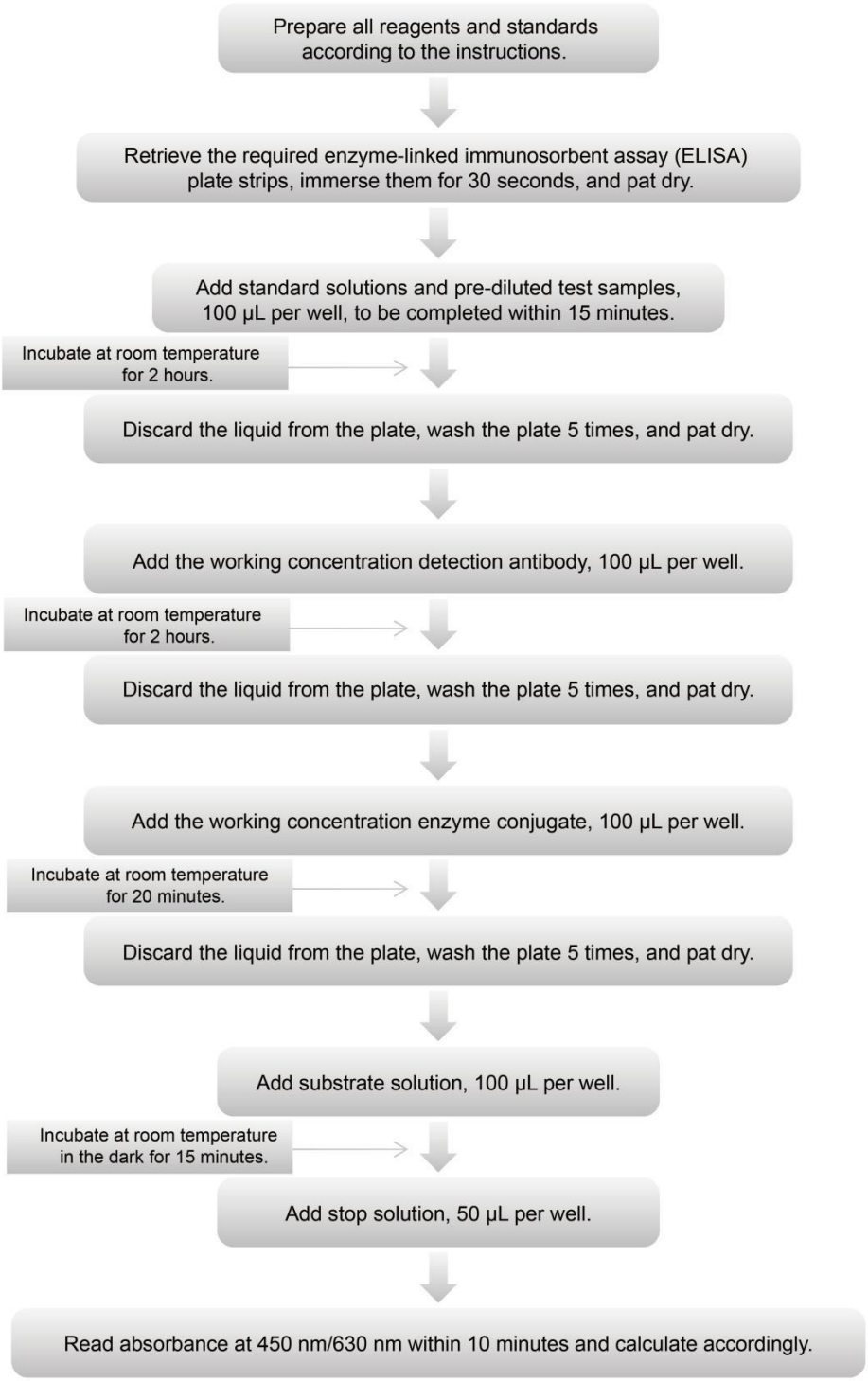
n.d. indicating that the sample concentration value is below the detection range of 7.8 pg/mL.

7. Specificity Assay

This assay kit identifies both natural and recombinant human interleukin 6 (IL-6). Specific evaluations were conducted for the following factors, and no significant cross-reactivity or interference effects were observed.

Recombinant human:		Recombinant Mouse:	
IL-1α	IL-6R	IL-1β	
IL-1β	gp 130	IL-2	
IL-2	LIF	IL-4	
IL-4	OSM	IL-6	
IL-5	CNTF	IL-10	
IL-8		IL-12	
IL-10			
IL-11			
IL-12			

Detection Schematic



Frequently Asked Questions

Issues	Causes	Solution
Poor standard curve	Inaccurate pipetting volumes	Check the pipette, calibrate it regularly, handle it carefully, tightly cap the tip while mixing thoroughly, and minimize foam formation.
	Inappropriate dilution methods	
Low color intensity	Insufficient incubation time	Allow sufficient incubation time, and replace samples and reconstituted standards overnight.
	Inadequate pipetting volumes or improper dilution	Calibrate pipettes and standardize operations.
High coefficient of variation (CV)	Improper washing of the ELISA plate	Use the correct washing procedure; if using a plate washer, check all ports for blockages.
	Contaminated washing solution	Prepare fresh washing solution.
Low sensitivity	Improper storage of the assay kit	Store according to the product component table.