Human IL-8 ELISA Kit

Product description

Human IL-8 ELISA Kit is an in vitro enzyme-linked immunosorbent assay kit used for the quantitative determination of Human IL-8 (Interleukin-8) in serum and cell culture supernatants. Specific antibodies against Human IL-8 are pre-coated on high-affinity enzyme plates. Standard samples and test samples are added to the wells of the enzyme plate and, after incubation, the IL-8 present in the samples binds to the solid-phase antibody. After washing to remove unbound substances, a detection antibody is added and incubated to bind. Following another wash, an enzyme conjugate (Streptavidin-HRP) is added and incubated to bind. After washing, aTMB chromogenic substrate is added for color development in the absence of light. The intensity of the color reaction is proportional to the concentration of IL-8 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).

IL-8, also known as CXCL8, is produced by macrophages and epithelial cells. It is a member of the α or CXC chemokine family and has a molecular weight of 8-9 kDa. IL-8 is a chemotactic factor that can attract neutrophils, eosinophils, and T cells, but not monocytes. It also plays a role in neutrophil activation and is released from several cell types in response to inflammatory stimuli.

Specifications

Item Number	P162022S / P162022E
Specification	48 T / 96 T
Detection Range	7.81-500 pg/mL
Detection Method	Sandwich ELISA
Detection Species	Human
Detection Time	5 hours
Sensitivity	0.382 pg/mL
Dilution Linearity	83.1 - 109.8%
Recovery Rate	73.7 - 112.4%
Intra-assay Variability	8.4%
Inter-assay Variability	7.0%

Components

Component Number	Component Name	Storage Temperature	P162022S	P162022E
P162022-A	Plate	2~8°C	48 T	96 T
P162022-B	Standard	2~8°C	1 tube	2 tubes
P162022-C	Detection Antibody	2~8°C	60 µL	120 µL
P162022-D	Enzyme Conjugate	2~8°C(Avoid Light)	30 µL	60 µL
P162022-E	5x Dilution Buffer	2~8°C	12 mL	25 mL
P162022-F	20x Wash Buffer	2~8°C	25 mL	50 mL
P162022-G	Substrate Solution	2~8°C(Avoid Light)	8 mL	15 mL
P162022-H	Stop Solution	Room Temperature	5 mL	10 mL
P162022-I	Plate Sealers	Room Temperature	3 pieces	5 pieces

Storage

The assay kit can be stored at 2~8°C. Alternatively, the reagents can be stored according to the storage conditions provided in the component information to avoid contamination and repeated freeze-thaw cycles. Diluted working solutions should be used immediately and not reused. The shelf life is 1 year.

Table 1. Reagent Storage Table After Initial Use

Material Name	Storage Conditions
Plate	Unused strips can be returned to the aluminum foil bag, 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	
5x Dilution Buffer	Store at 2~8°C for 1 month, avoiding contamination.
20x 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 detection of Human IP-10 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, wear laboratory clothing and disposable gloves when operating.
3. The reagent kit should be used within the shelf life. It is prohibited to mix reagents from different batches.
4. This product can only be used to detect target antigens and samples as indicated in the instruction manual. Other applications require validation by the user, and the reliability and accuracy of the use should be evaluated based on the results.
5. Do not mix or substitute reagents or materials from different batches or suppliers of other kits.

Common Technical Tips

1. When the sample OD value is higher than the S1 OD value, further dilution should be performed in an appropriate sample diluent.
2. Avoid generating foam during mixing.
3. Replace tips promptly when adding standard samples, samples, and others to avoid cross-contamination.
4. Ensure proper sealing of the ELISA plate or complete coverage with plate seal film during incubation.
5. Please completely remove all solutions and buffers during the washing steps.
6. Before dissolving the standard samples, do not invert the standard sample tube randomLy. After inverting the standard sample tube, thoroughly mix it up and down after adding the buffer, then centrifuge at low speed.
7. During the experiment, place the reagents according to the instructions.
8. Promptly discard buffers after the experiment; use immediately.
9. Different products have different components in the reagent kit and cannot be used interchangeably.

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, then test immediately or aliquot and store at -20°C or below.

2) Serum Samples: Collect serum in tubes free of pyrogens and endotoxins. After clotting for 30 minutes, centrifuge at 1, 000×g for 10 minutes. Test immediately after aspirating serum samples or aliquot and store at -20°C or below.

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

[Note]Visible precipitates in the sample must be removed before testing. Do not use samples with severe hemolysis or high lipid content. Samples should be aliquoted and stored at -20°C to prevent loss of IL-8 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 brought to room temperature (25°C ± 3°C) and gently mixed.

If sample dilution is required, use the specified sample dilution buffer for dilution.

Recommended dilutions for normal serum/plasma samples (for reference only): 1:9 dilution with sample diluent; for cell culture supernatant (for reference only): undiluted.

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

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

All reagent components and samples need to be brought to room temperature before use. To ensure the accuracy of the experiment, this should be done within 15 minutes before use.

1) 1×Wash Solution Preparation: Equilibrate concentrated solution to room temperature, fully dissolve without crystallization. Mix well, take 25 mL of 20× wash solution and dilute to 500 mL with distilled water; specific preparation volumes can be adjusted based on the amount used each time.

2) 1×Dilution Solution Preparation: Allow the concentrated solution to equilibrate to room temperature, ensuring complete dissolution without any crystallization. Mix thoroughly, then take 10 mL of the 5×dilution solution and transfer it to distilled water, followed by volumetrically adjusting to 50 mL. The specific volume for preparation can be tailored according to the required

quantity for each use. The 1× dilution solution is utilized for diluting standard substances, test samples, detecting antibodies, and enzyme conjugates.

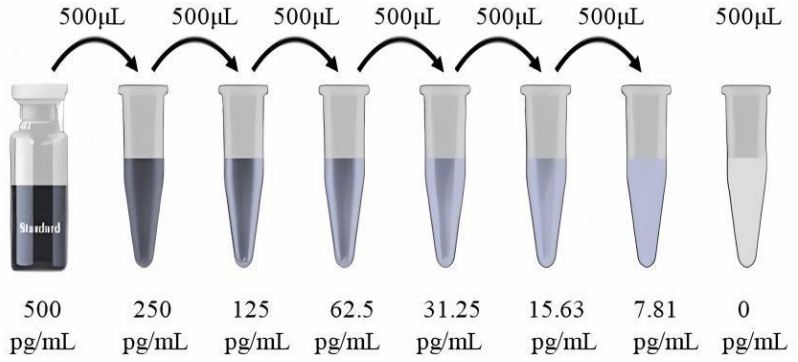
3) Detection Antibody Preparation: Centrifuge at 10000 rpm for 20 seconds before use, then dilute with antibody diluent at a ratio of 1:100 for working concentration, for example: take 60 μL and dilute to 6mL with antibody diluent; specific preparation volumes can be adjusted based on the amount used each time, mix thoroughly.

4) Enzyme Conjugate Preparation: Centrifuge at 10000 rpm for 20 seconds before use, then dilute with enzyme diluent at a ratio of 1:200 for working concentration, for example, take 30 μL and dilute to 6 mL with antibody/enzyme diluent; specific preparation volumes can be adjusted based on the amount used each time, mix thoroughly.

5) Preparation of Standard Curve: Prepare 7 sterile 1.5 mL centrifuge tubes and label them according to the standard concentrations. Preparation of S1: Dissolve one vial of standard lyophilized powder in the amount indicated by the label with sample diluent, mix thoroughly, labeled as 500 pg/mL. Add 500 μL of 1×sample diluent to each centrifuge tube, take 500 μL of S1 and mix thoroughly into the first centrifuge tube, then take 500 μL to the next labeled concentration tube and mix thoroughly, creating a 2-fold dilution standard curve, starting with the highest concentration labeled as 500 pg/mL and the lowest concentration as 7.81 pg/mL, according to the following preparation method. A standard curve should be prepared for each experiment, and standard curves from different kits or different times should not be mixed. For sample testing, 100 μL of each standard is required per well; ensure the preparation volume is greater than the required volume to avoid insufficient usage.

Table 2. Preparation of IL-8 Standard Curve (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	500	500	250
S3	500	500	125
S4	500	500	62.5
S5	500	500	31.25
S6	500	500	15.63
S7	500	500	7.81
Blank	500	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, diluted standards, and test samples.
2. ELISA Plate Determination: Calculate the number of ELISA plate wells needed for test samples and standards. Remove the ELISA plate strips from the aluminum foil bag, return the unused strips to the bag, seal the bag, and store at low temperature.
3. Soak the ELISA plate: Soak the ELISA plate in 1×wash solution (350 μL/well), discard the liquid from the wells after 30 seconds, and tap dry the ELISA plate. The liquid volume affects the test results significantly; ensure no residual wash solution remains after the final tapping.
4. Sample Incubation: Add various gradient standards and diluted test samples, 100 μL/well, ensuring spot sampling is completed within 15 minutes. Incubate at room temperature for 2 hours.
5. Plate Washing: Discard the liquid from the wells, wash the plate 5 times with 1×wash solution (350 μL/well), and tap dry the ELISA plate.
6. Detection Antibody Incubation: Add the detection antibody prepared to working concentration to the ELISA plate, 100 μL/well, and incubate at room temperature for 2 hours.
7. Plate Washing: Discard the liquid from the wells, wash the plate 5 times with 1×wash solution (350 μL/well), and tap dry the ELISA plate.
8. Enzyme Conjugate Incubation: Add the enzyme conjugate prepared to working concentration to the ELISA plate, 100 μL/well, and incubate at room temperature for 30 minutes.
9. Plate Washing: Discard the liquid from the wells, wash the plate 5 times with 1×wash solution (350 μL/well), and tap dry the ELISA plate.
10. Color Development: Before use, equilibrate the substrate solution to room temperature for 10 minutes. Add the substrate solution to the ELISA plate, 100 μL/well, and incubate at room temperature in the dark for 20 minutes.
11. Stop Solution: Add 50 μL/well of stop solution to the ELISA plate. At this point, the color changes from blue to yellow. Gently shake the ELISA plate to ensure uniform color development.
12. Reading: Read the absorbance values at 450 nm/630 nm within 10 minutes.

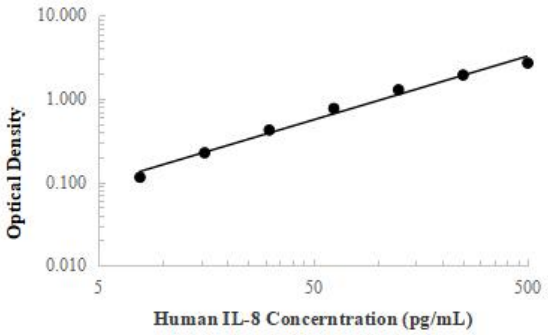
Standard Curve Establishment

Calculate the average OD values for duplicate wells of standards 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 standard concentrations as the x-axis and the logarithm of calibrated OD values as 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.

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 (pg/mL)	Absorbance		Mean Value	Calibration Value
500	2.799	2.618	2.708	2.690
250	1.917	1.983	1.950	1.931
125	1.370	1.233	1.302	1.283
62.5	0.811	0.757	0.784	0.766
31.25	0.442	0.436	0.439	0.421
15.625	0.252	0.234	0.243	0.225
7.81	0.134	0.131	0.133	0.114
0	0.021	0.016	0.019	/

2. Sensitivity Detection

The minimum detection limit of IL-8 is 0.382 pg/mL, calculated by averaging the zero well OD values from 20 repeated measurements and their standard deviation.

3. Precision Detection

Intra-assay Precision

Three samples of known concentrations were assayed 20 times on the same ELISA plate to evaluate

Inter-assay Precision

Three samples of known concentrations were assayed 30 times on different ELISA plates to evaluate.

Project	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
	20	20	20	30	30	30
Mean Value	14.0	73.6	251.6	13.5	71.0	249.4
Standard Deviation	1.25	5.49	22.26	0.87	5.01	18.69
Coefficient of Variation (%)	8.9	7.5	8.8	6.5	7.1	7.5

4. Recovery Rate Detection

The recovery of IL-8 was determined by adding IP-10 at different levels to the samples. The recovery rates are as follows:

Sample Types	Average Recovery Rate (%)	Range (%)
Serum	90.0	73.7-111.6
Cell culture supernatant	90.9	81.6-112.4

5. Dilution Linearity Test

Serum Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	90.1	83.5-99.7
1:04	88.3	87.0-89.0
1:08	91.6	83.7-98.2
1:16	89.2	83.1-95.2

Cell Culture Supernatant Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	99.5	90.6-106.6
1:04	99.1	90.7-109.8
1:08	98.1	92.2-106.1
1:16	99.9	95.1-108.5

6. Sample Values

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

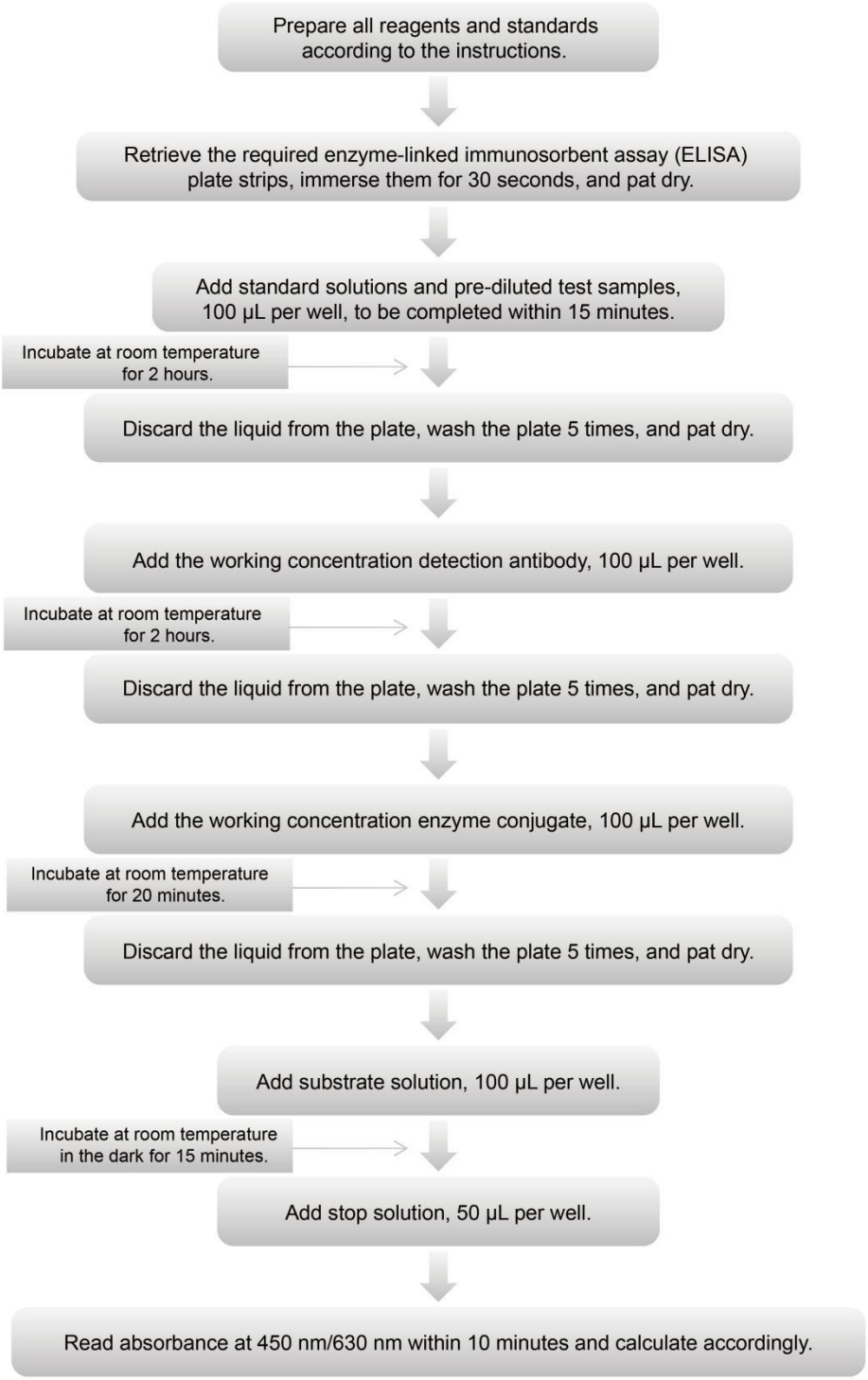
Sample Types	Sample Number	Mean Value (pg/mL)	Sample Value (pg/mL)
Serum	10	148.1	24.0-280.0
Cell Culture Supernatant	10	n.d.	n.d.

7. Specificity Assay

This assay kit recognizes both native and recombinant IL-8, with no observed significant cross-reactivity or interference effects.

Recombinant human:			Recombinant mouse:	Recombinant amphibian:	Natural proteins:
IL-1α	IL-6 R	TGF-α	IL-1α	TGF-β5	bovine FGF acidic
IL-1β	IL-7	TGF-β1	IL-3		bovine FGF basic
IL-1ra	IL-9	TGF-β3	IL-4		human PDGF
IL-2	IL-10	TGF-β RII	IL-5		porcine PDGF
IL-3	IL-11	TNF-α	IL-6		human TGF-β1
IL-4 R	IL-12	TNF-β	IL-9		porcine TGF-β1
IL-5	IL-13	TNF RI	IL-10		porcine TGF-β2
IL-5 Rβ	IP-10	TNF RII	IL-13		
IL-6	SCF	VEGF	TNF-α		

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.