Human IL-10 ELISA Kit

Product description

Human Interleukin-10 (Human IL-10), also known as Cytokine Synthesis Inhibitory Factor (CSIF), can inhibit the activation of Th1 cells and the production of related cytokines (such as IL-1β and TNF-α). IL-10 is a 178 amino acid molecule containing two intrachain disulfide bridges and is expressed as a non-covalently associated homodimer with a molecular weight of 36 kDa. Mature human IL-10 shares 72% to 86% amino acid sequence identity with IL-10 from cow, dog, horse, cat, mouse, sheep, pig, and rat. IL-10 can target various leukocytes, attenuating excessive immune responses. It affects three important functions of monocytes/macrophages: antigen presentation, release of immune mediators, and phagocytosis, representing its inhibition of all functions of monocytes/macrophages. Similar to IL-4 and IL-5, IL-10 is also involved in the development of autoimmune diseases. IL-10's presence can be observed in inflammatory bowel disease (IBD); it plays a protective role in chronic liver inflammation.

The Arcegen Human IL-10 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay kit used for the quantitative measurement of IL-10 in serum, plasma, and cell culture supernatants. Specific anti-IL-10 antibodies are pre-coated on a high-affinity enzyme-linked immunosorbent assay plate. Standard samples and test samples are added to the wells of the plate, and after incubation, IL-10 present in the samples binds to the solid-phase antibody. After washing to remove unbound substances, a detection antibody is added for incubation and binding, followed by washing. Then, a streptavidin-HRP enzyme conjugate is added for incubation and binding. After washing, aTMB color substrate is added for color development in the absence of light. The intensity of the color reaction is directly proportional to the concentration of IL-10 in the sample. The reaction is terminated by adding a stop solution, and the absorbance values are measured at 450 nm wavelength (reference wavelength 570-630 nm).

Specifications

Item Number	P162010S/P162010E
Specification	48 T/96 T
Detection Range	62.5-4000pg/mL
Detection Method	Sandwich ELISA
Detection Time	4.5hours
Sensitivity	27.5pg/mL
Dilution Linearity	79-117%
Recovery Rate	80-118%
Intra-assay Variability	5.9%

Inter-assay Variability	7.1%

Components

Component Number	Component Name	Storage Temperature	P162010S	P162010E
P162010-A	ELISA Plate	2~8°C	48 T	96 T
P162010-B	Standard	2~8°C	1 tube	2 tubes
P162010-C	Detection Antibody	2~8°C	120 µL	240 µL
P162010-D	Enzyme Conjugate	2~8°C(Avoid Light)	30 µL	60 µL
P162010-E	Sample Dilution Buffer	2~8°C	8mL	15 mL
P162010-F	Antibody/Enzyme Dilution Buffer	2~8°C	15 mL	30 mL
P162010-G	20x Wash Buffer	2~8°C	25 mL	50 mL
P162010-H	Substrate Solution	2~8°C(Avoid Light)	8mL	15 mL
P162010-I	Stop Solution	Room Temperature	5 mL	10 mL
P162010-J	Plate Sealant Film	Room Temperature	3 pieces	5 pieces

Storage

The kit can be stored at 2~8°C or according to the storage conditions specified for each component to avoid contamination and repeated freeze-thaw cycles. Diluted working concentration reagents should be prepared as needed and discarded after use; they should not be reused. The shelf life is 1 year.

Table 1. Reagent Storage Table After Initial Use

Component Name	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	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	
Sample Dilution Buffer	Store at 2~8°C for 1 month,avoiding contamination.
Antibody/Enzyme Dilution Buffer	
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 Sealant Film	

Instructions

1. Quantitative detection of IL-10 content in serum, plasma, and cell culture supernatant.
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 performed in an appropriate sample diluent.
2. Avoid generating foam during mixing.
3. When adding standards, samples, and others, timely replacement of tips is necessary to prevent cross-contamination.
4. Ensure proper sealing or covering of the plate during the incubation period.
5. Completely remove all solutions and buffers during the washing steps.
6. Before dissolving the standard, do not arbitrarily invert the standard vial. After inverting the standard vial, 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. Dispose of buffers promptly after completing the experiment; use once and discard.
9. Different kit components are not interchangeable and should not be used across different products.

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. Immediate detection or aliquot and store at -20°C or below.

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

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

[Note]Visible precipitates in the 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-10 activity. If testing within 24 hours, samples can be stored at 2~8°C to avoid repeated freeze-thaw cycles. Frozen samples should be slowly brought to room temperature (25°C ± 3°C) and gently mixed before testing.

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

Recommended dilution for normal serum/plasma samples (for reference only): 1:1 dilution with sample diluent for serum, plasma samples (for reference only): undiluted.

Due to variations in the target protein content in samples, the dilution ratio for each sample is recommended based on pre-experimental 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

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

1) 1 × Wash Buffer Preparation: Equilibrate the concentrate to room temperature, dissolve

completely without any crystallization. Mix well, take 25 mL of 20×Wash Buffer into distilled water, then bring the volume up to 500 mL; the specific volume can be prepared according to the amount needed for each use.

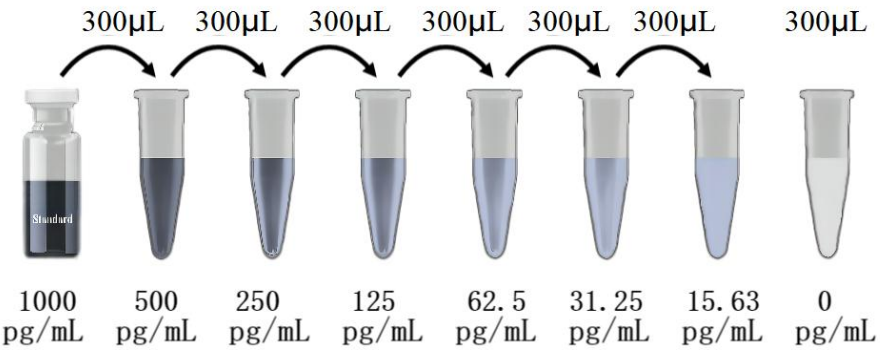
2) Detection Antibody Preparation: Before use, centrifuge at 10, 000 rpm for 20 seconds, then dilute the detection antibody to working concentration at 1:50 with antibody diluent, for example, take 120 μL and bring the volume up to 6 mL with antibody diluent; specific volume can be prepared according to the amount needed for each use, mix well.

3) Enzyme Conjugate Preparation: Before use, centrifuge at 10, 000 rpm for 20 seconds, then dilute the enzyme conjugate to working concentration at 1:200 with enzyme diluent, for example, take 30 μL and bring the volume up to 6 mL with antibody/enzyme diluent; specific volume can be prepared according to the amount needed for each use, mix well.

Preparation of Standard Curve: Prepare 7 sterile 1.5 mL microcentrifuge tubes, label them sequentially according to the standard concentrations. Preparation of S1: Dissolve an aliquot of standard freeze-dried material in sample diluent to the labeled amount, mix well, indicating it as 4000 pg/mL. Add 300 μL of 1×sample diluent to each tube, add 300 μL of S1 to the first tube, mix well, then transfer 300 μL to the next tube with the next concentration label, mix well, and continue this 2-fold dilution series. The starting highest concentration is labeled as 4000 pg/mL, and the lowest concentration is 62.5 pg/mL, following the preparation method below. Prepare a standard curve for each assay, and do not mix standard curves from different assay kits or different times. For sample testing, 100 μL of standard is needed per well, ensure the prepared volume is sufficient to avoid insufficient usage.

Table 2. IFN-γ Standard Curve Preparation (31.25 - 2000 pg/mL)

Standard Curve	Diluent (μL)	Volume of Standard Added (μL)	Final Concentration of Standard (pg/mL)
S1	As labeled	/	4000
S2	300	300	2000
S3	300	300	1000
S4	300	300	500
S5	300	300	250
S6	300	300	125
S7	300	300	62.5
Blank	300	0	0



Operating Instructions

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

1. Reagent Preparation: Prepare various reagents for testing, including diluted standard samples and samples under test.
2. Plate Determination: Calculate the number of plates needed for test samples and standards. Remove the plate from the aluminum foil bag, return the remaining plates to the bag, seal the bag, and store at low temperature.
3. Plate Soaking: Add 350 μL of 1× washing solution per well to soak the plate. After standing for 30 seconds, remove the liquid from the wells and tap-dry the plate. The volume of liquid is crucial for the test results, so ensure that there is no residual washing solution after the final tapping.
4. Sample Incubation: Add various concentrations of standard samples and appropriately diluted samples under test (100 μL per well). Ensure all samples are added within 15 minutes and incubate at room temperature for 2 hours.
5. Plate Washing: Discard the liquid from the wells, wash the plate 5 times with 350 μL of 1× washing solution per well, and tap-dry the plate.
6. Antibody Incubation: Add the detection antibody, pre-diluted to the working concentration, to the microplate strips (100 μL per well). Incubate at room temperature for 2 hours.
7. Plate Washing: Discard the liquid from the wells, wash the plate 5 times with 350 μL of 1× washing solution per well, and tap-dry the plate.
8. Enzyme Conjugate Incubation: Add the enzyme conjugate, pre-diluted to the working concentration, to the microplate strips (100 μL per well). Incubate at room temperature for 20 minutes.
9. Plate Washing: Discard the liquid from the wells, wash the plate 5 times with 350 μL of 1× washing solution per well, and tap-dry the plate.
10. Color Development: Allow the substrate solution to equilibrate to room temperature for 10 minutes before adding 100 μL per well to the microplate strips. Incubate at room temperature in the dark for 15 minutes.

11. Termination: Add 50 µL per well of stop solution to the microplate strips. The color will change from blue to yellow. Gently shake the microplate strips to ensure uniform color development.
12. Reading: Read the absorbance values at 450 nm and 630 nm within 10 minutes.

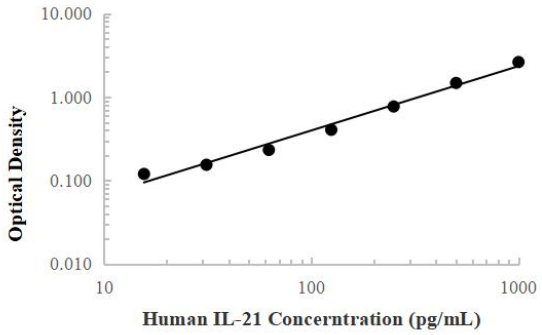
Standard Curve Establishment

Calculate the average OD values of duplicate wells for both standard samples and samples under test, and subtract the average OD value of blank wells to obtain the calibrated OD values. Plot the standard curve with the logarithm of the standard sample concentration as the x-axis and the logarithm of the calibrated OD values as 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 yield satisfactory results. The choice of method should be based on the specific experimental data analysis.

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
4000	2.192	2.339	2.265	2.269
2000	1.449	1.476	1.462	1.447
1000	0.791	0.823	0.807	0.826
500	0.453	0.436	0.444	0.450
250	0.259	0.261	0.260	0.251
125	0.177	0.173	0.175	0.155
62.5	0.111	0.109	0.110	0.109
0	0.055	0.051	0.053	/

2. Sensitivity Detection

The minimum detection limit of IL-10 is 27.5 pg/mL, calculated by repeating the measurement of the blank OD value 20 times to determine the mean and standard deviation.

3. Precision Detection

Intra-assay Precision

Three samples of known concentrations were assayed in duplicate for six times within the same ELISA plate to evaluate the intra-assay precision.

Inter-assay Precision

Three samples of known concentrations were assayed in duplicate for 18 times across different ELISA plates to evaluate the inter-assay precision.

Sample Types	Average Recovery Rate (%)			Range (%)		
Sample	1	2	3	1	2	3
	6	6	6	18	18	18
Mean Value	2228.9	545.8	133.4	2200.5	537.4	126.9
Standard Deviation	191.2	18.6	7.8	193.7	20.8	11.0
Coefficient of Variation (%)	8.6	3.4	5.8	8.8	3.9	8.6

4. Recovery Rate Detection

Recovery rates were determined by adding IL-10 at different levels to the samples, and the recovery rates are as follows:

Sample Types	Average Recovery Rate (%)	Range (%)
Serum	89.3	79.9-96.8
Plasma	105	85.5-115.1
Cell culture supernatant	106.3	83.8-117.6

5. Dilution Linearity Test

Serum Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	109.1	100.9-117.3
1:04	99.6	88.5-111
1:08	104.3	98.7-114.5
1:16	100.3	85.2-115.5

Plasma Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	97.8	93.6-102.5
1:04	96.6	90-101.3
1:08	93.0	78.9-102.7
1:16	90.8	81.4-97.3

Cell Culture Supernatant Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	101.8	98.3-104.4
1:04	89.2	83.8-101.3
1:08	98.6	85.1-113.7
1:16	89.8	81.2-97.1

6. Sample Values

Applying this assay kit, samples from several healthy volunteers are tested, with no detailed medication history available for the volunteers.

Sample Types	Sample Number	Mean Value(pg/mL)	Sample Value(pg/mL)
Serum	4	n.d.	n.d.
Plasma	8	n.d.	n.d.
Cell Culture Supernatant	6	n.d.	n.d.

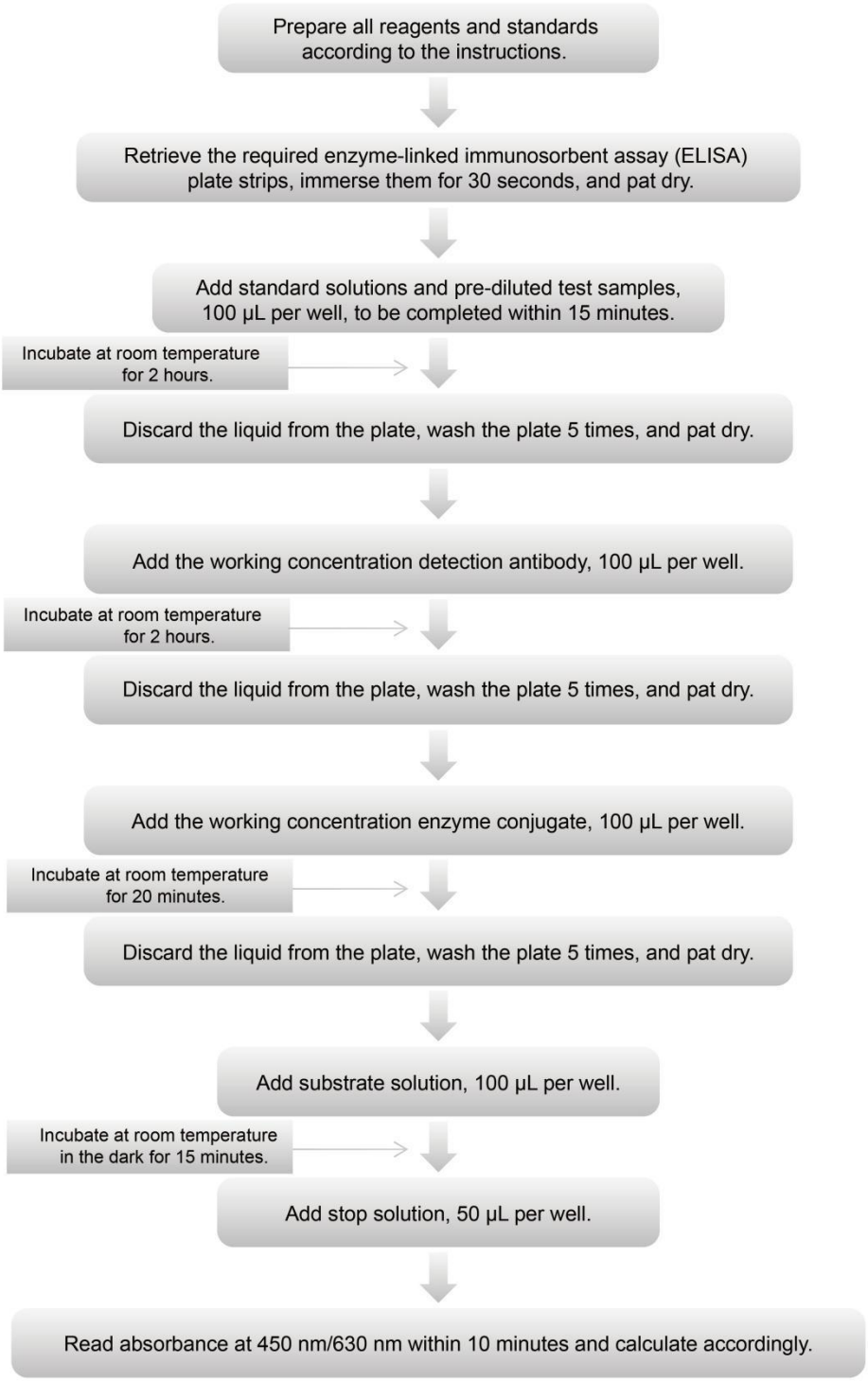
n.d. refers to samples with concentrations below the detection range of 62.5 pg/mL.

7. Specificity Assay

This assay kit identifies both natural and recombinant IL-10, with no observed significant cross-reactivity or interference.

Recombinant human		Recombinant Mouse:	Recombinant Rat:
IL-1β	IL-17A	IL-1β	IL-1β
IL-2	IL-21	IL-2	IL-2
IL-4	IL-22	IL-4	IL-4
IL-5	IL-23	IL-6	IL-6
IL-6	TNF-α	IL-10	IL-10
IL-8		IL-17A	IL-17A
IL-12		TNF-α	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.