Human IL-21 ELISA Kit

Product description

Human IL-21 ELISA (Enzyme-Linked Immunosorbent Assay) Kit is an in vitro enzyme-linked immunosorbent assay kit used for quantitative determination of IL-21 in serum, plasma, and cell culture supernatants. Specific anti-IL-21 antibodies are pre-coated onto high-affinity enzyme plates. Standards and samples are added to the wells of the enzyme plate, and after incubation, IL-21 present in the sample binds to the solid-phase antibody. After washing to remove unbound substances, a detection antibody is added for binding and incubated, followed by washing, and then the enzyme complex (Streptavidin-HRP) is added for binding and incubated. After washing, a colorimetric substrateTMB is added for color development, avoiding light. The intensity of the color reaction is proportional to the concentration of IL-21 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).

Human Interleukin-21 (IL-21) is a type I cytokine that regulates the functions of T cells, B cells, NK cells, and myeloid cells. IL-21 is produced by activated follicular helper T cells (Tfh), Th17 cells, and NKT cells. IL-21 derived from Tfh cells plays a crucial role in the development of humoral immunity through its autocrine effects on Tfh cells and paracrine effects on immunoglobulin maturation, plasma cell differentiation, and memory B cells. Mature human IL-21 consists of 133 amino acids and shares 63% and 61% sequence identity with mouse and rat IL-21, respectively. IL-21 is closely associated with clinical conditions such as cancer immunotherapy, viral infections, and allergies. It also inhibits skin hypersensitivity reactions by limiting the production of allergen-specific IgE and degranulation of mast cells.

Specifications

Item Number	P162019S / P162019E
Specification	48 T / 96 T
Detection Range	15.63-1000 pg/mL
Detection Method	Sandwich ELISA
Detection Species	Human
Detection Time	4.5 hours
Sensitivity	8.16 pg/mL
Dilution Linearity	81 - 117%
Recovery Rate	76 - 121%
Intra-assay Variability	3.3%
Inter-assay Variability	5.0%

Components

Component Number	Component Name	Storage Temperature	P162019S	P162019E
P162019-A	Plate	2~8°C	48 T	96 T
P162019-B	Standard	2~8°C	1 tube	2 tubes
P162018-C	Detection Antibody	2~8°C	120 µL	240 µL
P162019-D	Enzyme Conjugate	2~8°C(Avoid Light)	30 µL	60 µL
P162019-E	5x Dilution Buffer	2~8°C	8 mL	15 mL
P162019-F	20x Wash Buffer	2~8°C	25 mL	50 mL
P162019-G	Substrate Solution	2~8°C(Avoid Light)	8 mL	15 mL
P162019-H	Stop Solution	Room Temperature	5 mL	10 mL
P162019-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. Used for quantitative detection of IL-6 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, 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.

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 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-21 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): 1:1 dilution with sample diluent; for cell culture supernatant (for reference only): undiluted.

Due to variations in the content of target protein 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: Equilibrate the concentrated solution to room temperature,

ensuring complete dissolution without crystallization. Mix well, take 10 mL of 5x dilution solution and add it to distilled water, then bring the volume up to 50 mL; the specific volume for preparation can be adjusted based on the amount needed for each use. The 1x dilution solution is used to dilute standard substances, test samples, detect antibodies, and enzyme conjugates.

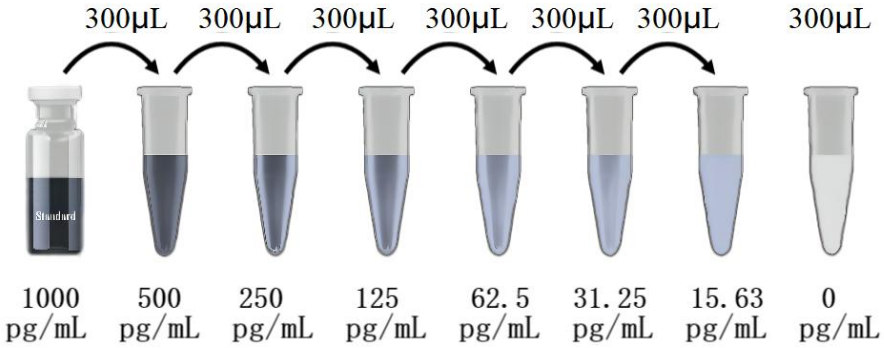
3) Detection Antibody Preparation: Centrifuge at 10000 rpm for 20 seconds before use, then dilute with antibody diluent at a ratio of 1:50 for working concentration, for example: take 120 μL and dilute to 6mL with antibody diluent; specific preparation volumes can be adjusted based on the amount used 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 6mL 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 1000 pg/mL. Add 300 μL of 1×sample diluent to each centrifuge tube, take 300 μL of S1 and mix thoroughly into the first centrifuge tube, then take 300 μL to the next labeled concentration tube and mix thoroughly, creating a 2-fold dilution standard curve, starting with the highest concentration labeled as 1000 pg/mL and the lowest concentration as 15.63 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-21 Standard Curve (15.63-1000 pg/mL)

Standard Curve	Diluent (μL)	Volume of Standard Added (μL)	Final Concentration of Standard (pg/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, 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 20 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 15 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.

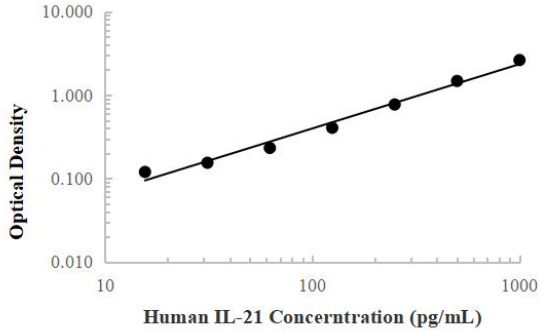
Standard Curve Establishment

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
1000	2.688	2.562	2.625	2.625
500	1.522	1.428	1.475	1.477
250	0.806	0.745	0.776	0.771
125	0.403	0.395	0.399	0.406
62.5	0.240	0.233	0.236	0.233
31.25	0.159	0.152	0.156	0.155
15.625	0.122	0.116	0.119	0.120
0	0.075	0.070	0.073	/

2. Sensitivity Detection

The minimum detection limit of IL-21 is 8.16 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 10 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
	10	10	10	30	30	30
Mean Value	810.2	282.4	17.5	818.2	274.9	16.6
Standard Deviation	21.1	3.7	1.1	21.7	7.2	1.6
Coefficient of Variation (%)	2.6	1.3	6.1	2.7	2.6	9.8

4. Recovery Rate Detection

The recovery of IL-21 was determined by adding IL-21 at different levels to the samples. The recovery rates are as follows:

Sample Types	Average Recovery Rate (%)	Range (%)
Serum	104.0	94.5-119.4
Plasma	85.5	76.8-91.8
Cell culture supernatant	105.0	82.8-121.8

5. Dilution Linearity Test

Serum Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	104.7	104.2-105.0
1:04	101.3	100.1-101.9
1:08	112.5	108.0-117.8
1:16	113.6	112.3-114.9

Plasma Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	99.3	97.1-101.3
1:04	93.9	93.0-95.3
1:08	92.3	90.6-93.1
1:16	87.0	81.3-91.6

Cell Culture Supernatant Dilution Ratio	Mean Expected Value (%)	Range (%)
1:02	109.6	108.4-111.8
1:04	107.8	107.3-108.1
1:08	105.9	100.2-110.5
1:16	96.9	93.3-100.0

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	3.7	n.d.-36.9
Plasma	10	2.2	n.d.-21.7
Cell Culture Supernatant	6	n.d.	n.d.

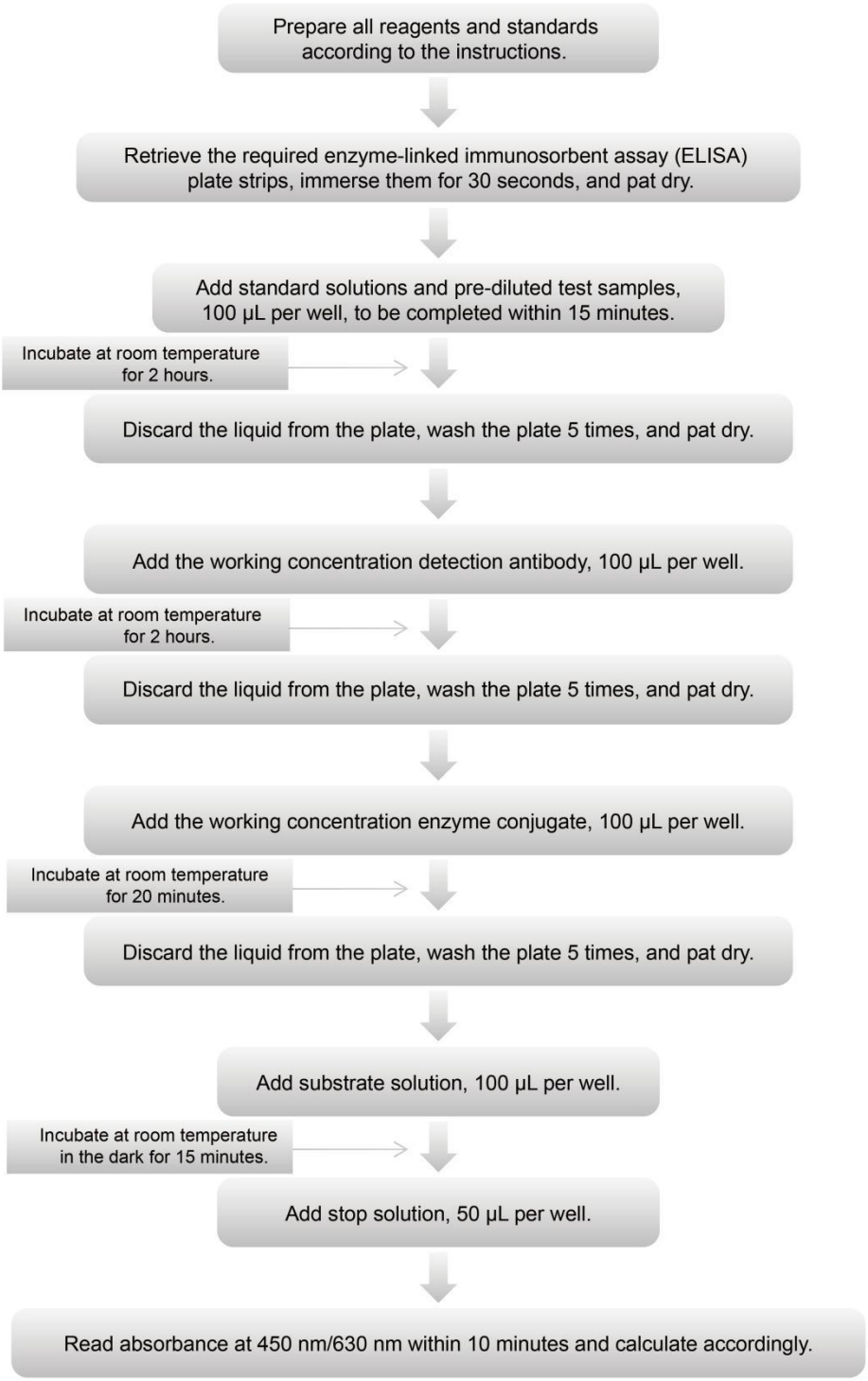
n.d. Refers to sample concentrations below the detection range of 15.63 pg/mL

7. Specificity Assay

This assay kit recognizes both natural and recombinant IL-21, with no observed significant cross-reactivity or interference effects.

Recombinant Human:		Recombinant Mouse:	Recombinant Rat:
IFN-γ	IL-10	IFN-γ	IFN-γ
IL-1β	IL-12	IL-1β	IL-1β
IL-2	IL-17	IL-2	IL-2
IL-4	IL-18	IL-4	IL-4
IL-5	IL-22	IL-6	IL-6
IL-6	MCP-1	IL-10	IL-10
IL-8	TNF-a	TNF-a	TNF-a

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.