Human IL-10 ELISA Kit

Please read the manual carefully before use.

Cat. No. NE102

Storage: at 2~8°C in the dark for one year.

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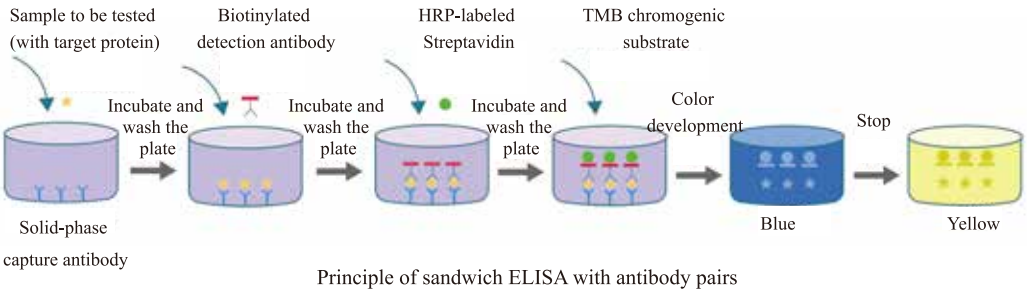
Description

Interleukin-10 (IL-10), also known as cytokine synthesis inhibitory factor (CSIF), is a pleiotropic cytokine that can exert immunosuppressive or immunostimulatory effects in various types of cells. IL-10 is a homodimeric secretion, mainly produced by monocytes, T cells (mainly Th1 cells), B cells, NK cells, and macrophages. IL-10 has broad anti-inflammatory activity and inhibits the expression of many pro-inflammatory factors, chemokines, and chemokine receptors. IL-10 inhibits the production of IFN-γ and IL-2 by Th1 lymphocytes and the production of IL-4 and IL-5 by Th2 lymphocytes. IL-10 can also inhibit the expression of cell surface antigens, such as CD23, CD80, CD86, etc. This kit uses Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) to measure the amount of IL-10 in human serum, plasma, and cell culture supernatant. The ELISA microplate in the kit is precoated with a high-affinity anti-human IL-10 antibody. The standard or test sample is added to the microplate wells. After incubation, IL-10 present in the sample will be bound specifically to the precoated antibody on the microplate wells. After washing, biotin-labeled anti-human IL-10 detection antibody is added to the microplate wells. After re-incubation, the detection antibody will be bound specifically to IL-10 anchored on the microplate wells. Subsequently, the horseradish peroxidase-labeled streptavidin (Streptavidin-HRP) is added to the microplate wells and incubated. Biotin and streptavidin on the detection antibody produces the "coated antibody-human IL-10 protein-detection antibody-Streptavidin-HRP" immune complex through high-strength non-covalent binding. After washing again, the chromogenic substrate TMB is added to the wells. HRP catalyzes the TMB substrate to create blue coloration, with intensity positively correlated with the concentration of IL-10 in the sample. The reaction is stopped by adding stop solution, and the absorbance is measured at 450 nm (reference wavelength 570-630 nm). A standard curve is drawn to calculate the concentration of IL-10 in the sample based on the absorbance value. This kit is highly specific, has high detection sensitivity and is convenient to use.



Principle of sandwich ELISA with antibody pairs

Suitable Sample Types

Cell culture supernates, serum, plasma, etc.

Kit Contents

Component	NE102-01	Storage
Human IL-10 Antibody Precoated ELISA Plate	96 T	at 2~8°C
Human IL-10 Standard	2 bottles	at 2~8°C
Standard & Sample Diluent	15 ml/bottle	at 2~8°C
100× Human IL-10 Detection Antibody	120 µl/test	at 2~8°C
100×Streptavidin-HRP	120 µl/test	at 2~8°C in the dark
Detection Antibody & Streptavidin-HRP Diluent	25 ml/bottle	at 2~8°C
20× Wash Buffer	50 ml/bottle	at 2~8°C
TMB Chromogenic Substrate	12 ml/bottle	at 2~8°C in the dark
Stop Solution	6 ml/bottle	at 2~8°C
Plate Sealers	4 pieces	

Note: The kit can be stored at 2~8°C for 1 month when it was opened; please use the unopened kit within 1 year.

Self-prepared Regents and Equipment

1. Microplate reader: at a main wavelength of 450 nm, a reference wavelength of 620 nm.
2. Deionized water.
3. EP tubes, pipettes, tips, graduated cylinders, etc.
4. Microplate shaker.
5. Automatic plate washer or 8-channel manual plate washeror multi-channel pipette.

Sample Collection

1. Cell culture supernatant: Collect the cell culture supernatant and centrifuge it at 300 × g for 10 minutes at 4°C. Aliquot the supernatant into EP tubes and store at -20°C. Avoid repeated freeze-thaw cycles. For detection within 24 hours, it can be stored at 2~8°C.
2. Serum: After the blood has clotted for 30 minutes at room temperature, centrifuge it at 1000 × g for 10 minutes at 4°C. Then, divide the supernatant into EP tubes and store at -20°C. Avoid repeated freeze-thaw cycles. For detection within 24 hours, it can be stored at 2~8°C.
3. Plasma: EDTA or sodium citrate is recommended as anticoagulants. Collect the whole blood into blood collection tubes containing anticoagulant and mix it. After placing it for at least 20 minutes at room temperature, centrifuge it at 1000 × g for 10 minutes at 4°C. Aliquote the supernatant into EP tubes and store it at -20°C. Avoid repeated freeze-thaw cycles. For detection within 24 hours, it can be stored at 2~8°C.

Note: Serum and plasma samples with hemolysis and hyperlipidemia may affect the accuracy of test results and should be avoided.

Sample Dilution

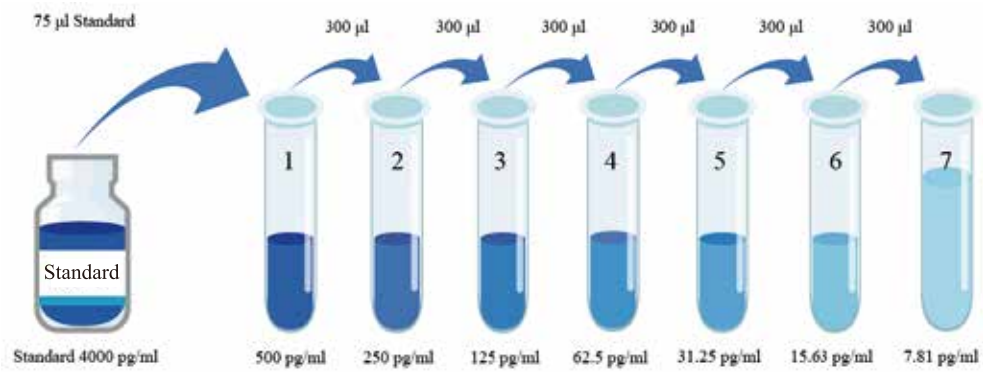
1. Serum/plasma samples need to be diluted 2-fold with standard & sample diluent, for example: 100 µl serum + 100 µl standard & sample diluent.
2. If the positive value of the cell culture supernatant sample is within the range of the curve, it does not need to be diluted with the diluent, and can be directly detected with the original solution; if the positive value exceeds the range of the curve, it needs to be diluted to the curve range with the diluent for detection. The concentration should be multiplied by the corresponding dilution factor.

3. It is recommended to perform a pre-experiment to determine the dilution factor before the formal experiment.

Working Solution Preparation

Bring all reagents to room temperature before use. If crystals appear in the 20× Wash Buffer, put them in a water bath at 37°C until all the crystals have dissolved.

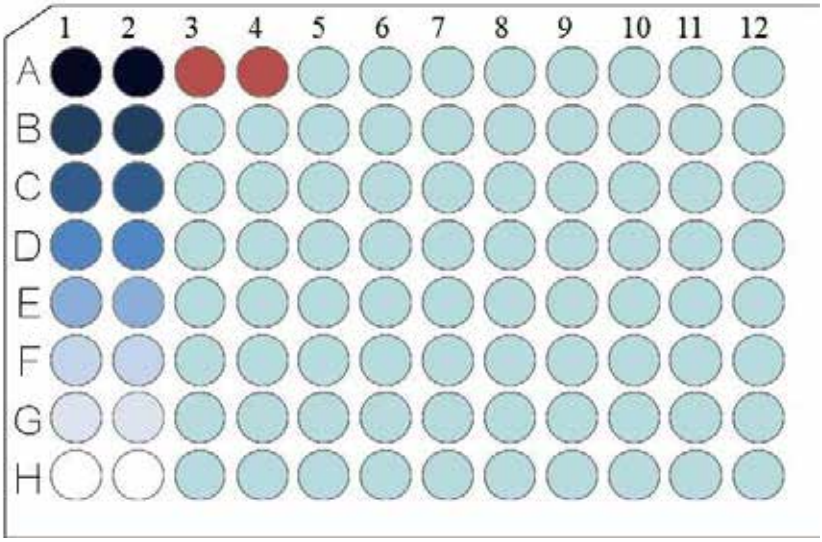
1. **1×Human IL-10 Detection Antibody**: Spin and centrifuge the tube before use to collect the liquid on the tube wall at the bottom. Dilute 100× human IL-10 detection antibody to 1×working concentration with Detection Antibody & Streptavidin-HRP Diluent according to the amount required for the current experiment. Use within 15 minutes after preparation.
2. **1×Streptavidin-HRP**: Spin and centrifuge the tube before use to collect the liquid on the tube wall at the bottom. Dilute 100× Streptavidin-HRP to 1× working concentration with Detection Antibody & Streptavidin-HRP Diluent according to the amount required for the experiment. Use within 15 minutes after preparation.
3. **1×Wash Buffer**: Dilute the 20×washing solution to 1×working concentration with deionized water according to the amount required for the current experiment. After preparation, it can be stored at 2~8°C for 30 days.
4. **Dissolve Human IL-10 Standard**: Dissolve the lyophilized standard with the corresponding volume of standard & sample diluent according to the dissolution volume indicated on the standard label. The concentration of the reconstituted standard is 4000 pg/ml. Please use the redissolved standard within 30 minutes.
5. **Standard Gradient Dilution**: Dilute the redissolved standard 8 times with Standard & Sample Diluent, take 75 µl of the reconstituted standard, add it to 525 µl standard & sample diluent, mix well, record it as a tube 1, and the concentration of tube 1 at this time is 500 pg/ml; then perform a 2-fold gradient dilution according to the figure below, and add 300 µl of standard & sample diluent to tubes 2-7 respectively; take 300 µl of the liquid from the No. 1 tube and add it to the tube 2. At this time, the concentration of the tube 2 is 250 pg/ml. After mixing, take 300 µl and add it to the tube 3. At this time, the concentration of the tube 3 is 125 pg/ml; and so on for gradient dilution to tube 7 (7.81 pg/ml). 500 pg/ml as the highest point of the standard curve, standard & sample dilution as the zero point (0 pg/ml) of the standard curve, that is, the blank value.



Experimental Protocol

1. Bring all reagents to room temperature before use. Take out the plates required for the current experiment, put the unused plates in an aluminium foil pouch in time to reseal, and store them at 2~8°C.
2. Add the diluted standard and sample to wells of the plate respectively, 100 µl/well, shake on a microplate shaker for 30 seconds to mix well, cover the plate and incubate for 2 hours at room temperature. It is recommended that the standards and samples be tested in duplicate, and the order of adding reagents should be consistent so that the results of each duplicate well test are consistent.
3. Discard the liquid in the wells and wash wells the plate with 1× washing solution, 300 µl/well. It is recommended to shake the plate on a microplate shaker for 30 seconds before washing, and then discard the washing solution. Repeat 5 times, the last time pat dry on a paper towel.
4. Add 1× Detection Antibody to each well, 100 µl/well, shake on a microplate shaker for 30 seconds to mix well, cover the plate with a sealing film, and incubate for 1 hour at room temperature.
5. Repeat Step 3.
6. Add 1× Streptavidin-HRP to each well, 100 µl/well, shake on a microplate shaker for 30 seconds to mix well, cover the plate with sealing film, and incubate for 30 minutes at room temperature .
7. Repeat Step 3.
8. Add TMB Chromogenic Substrate to each well, 100 µl/well, shake on a microplate shaker for 30 seconds to mix well, cover the plate with a sealing film, and incubate for 20 minutes at room temperature.
9. After the incubation, add stop solution, 50 µl/well, and read absorbance of the plate at the wavelength of 450 nm for the main wavelength and 620 nm for the reference wavelength.
10. After the experiment, put the unused reagents and the outer frame of the ELISA plate back into the kit and store them at 2~8°C. It is recommended to use up within 1 month.

Sample Loading on The Microtiter Plate



Notes: A1/A2: 100 µl 500 pg/ml Standard
B1/B2: 100 µl 250 pg/ml Standard
C1/C2: 100 µl 125 pg/ml Standard
D1/D2: 100 µl 62.5 pg/ml Standard
E1/E2: 100 µl 31.25 pg/ml Standard
F1/F2: 100 µl 15.63 pg/ml Standard
G1/G2: 100 µl 7.81 pg/ml Standard
H1/H2: 100 µl 0 pg/ml Standard (Standard & Sample Diluent)
A3/A4: 100 µl Sample

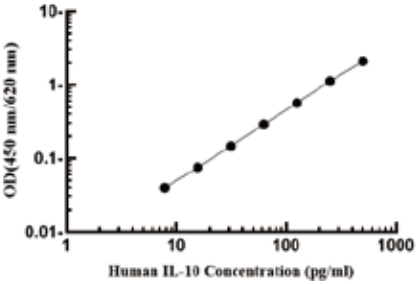
Result Analysis

1. Double-wavelength detection was performed with a microplate reader, and the OD values at the main wavelength of 450 nm and the reference wavelength of 620 nm were determined. The OD value is the OD measurement at 450 nm minus the OD measurement at 620 nm.
2. Calculate the average OD value of duplicate wells of the standard, then subtract the blank value (average OD value of the 0 pg/ml standard) to obtain the corrected value for the standard. Taking the concentration of the standard product as the abscissa and the OD correction value as the ordinate, the standard curve was generated by linear regression or the four-parameter method.
3. Calculate the sample concentration from the sample OD value and the standard curve equation. If the OD value of the sample is higher than the upper limit of the standard curve, it should be re-measured after appropriate dilution, and the corresponding dilution should be multiplied when calculating the concentration.

Reference Data

A standard curve needs to be established with each assay, and the following data are only for demonstration purposes.

Standard (pg/ml)	OD Value		Average	Corrected
500	2.179	2.021	2.1000	2.090
250	1.173	1.101	1.1370	1.127
125	0.588	0.565	0.5765	0.566
62.5	0.307	0.295	0.3010	0.291
31.25	0.160	0.155	0.1575	0.147
15.63	0.087	0.084	0.0855	0.075
7.81	0.051	0.049	0.0500	0.040
0	0.011	0.010	0.0105	0



Interpretation of the results of the standard duplicate wells: In the above table, the average OD value of the two duplicate wells of the 0 pg/ml standard is (0.011+0.010)/2=0.0105, and the correction value is set as 0. The average OD value of the two duplicate wells of the 500 pg/ml standard is (2.179+2.021)/2=2.1000, and the corrected value is 2.1000-0.0105=2.090.

Precision

Intra-assay Precision

Intra-assay precision was assessed by assaying 20 replicate wells on one microplate using 3 samples of known concentration.

Inter-assay Precision

Inter-assay precision was assessed by assaying 20 replicate wells on different microplate with 3 samples of known concentration.

	Intra-assay			Inter-assay		
	1	2	3	1	2	3
Average (pg/ml)	201.9	101.6	48.1	208.3	106.5	48.0
Standard Deviation	6.4	2.0	1.8	3.7	3.9	2.5
Coefficient of Variation (%)	3.2	2.0	3.7	1.8	3.6	5.3

Recovery Rate

Different concentrations of IL-10 were added to the serum, plasma and cell culture supernate of 4 healthy people, and the samples without IL-10 were used as the background to calculate the recovery rate.

Sample	Average Recovery (%)	Range (%)
Serum	98	88~102
EDTA Plasma	102	93~113
Sodium Citrate Plasma	95	84~103
Heparin Plasma	94	86~105
Cell Supernate	97	85~108

Linearity

High concentrations of human IL-10 were added to the serum and plasma of 4 healthy people, and linear dilution was performed to detect the linear recovery rate.

Sample Dilution	Recovery Rate (%)	Serum	EDTA Plasma	Sodium Citrate Plasma	Heparin Plasma
1:2	Average (%)	94	96	89	90
	Range (%)	89~97	87~100	85~94	87~96
1:4	Average (%)	99	103	94	92
	Range (%)	96~103	91~107	86~101	87~99
1:8	Average (%)	100	106	99	94
	Range (%)	97~104	102~112	96~102	91~98
1:16	Average (%)	100	103	98	98
	Range (%)	97~102	97~110	88~103	94~105

Calibration

The standard of this kit is high-purity recombinant human IL-10 calibrated by TransGen Biotech.

Sensitivity

The lowest detectable concentration of human IL-10 was 3.5 pg/ml. Sensitivity is the corresponding concentration calculated from the mean of 20 replicate zero standard OD values plus two standard deviations.

Sample Value

The level of IL-10 in 30 healthy human serum samples was detected by this kit, and the detection values of 30 samples were all lower than 7.81 pg/ml.

Specificity

This kit recognizes native and recombinant human IL-10. Using recombinant human EGF, FGF-basic, GM-CSF, IFN-γ, IL-1β, IL-2, IL-6, IL-12 p70, IL-12B, IL-23, TNF-α and other cytokines for specific evaluation, no cross-reactivity and interference effects were observed.

Notes

1. This kit should be stored at 2~8°C in the dark and used up within 1 month after opening.
2. To ensure accurate results, a standard curve is required for each assay..
3. All reagents used in the experiment should be thoroughly mixed.
4. After each plate washing, pat dry on a paper towel. If there are air bubbles in the plate wells, use a pipette tip to puncture them. Note that only one pipette tip can be used in each well to avoid cross-contamination.
5. TMB chromogenic substrate is a colorless and transparent liquid, please do not use it if there is discoloration.
6. After TMB develops color, it can be judged whether it is necessary to add a stop solution in advance or later according to the depth of color development.
7. After adding the stop solution, read within 30 minutes.
8. It is recommended to use the main wavelength of 450 nm and the reference wavelength of 620 nm for reading. If only a single wavelength of 450 nm is used for reading, the overall OD value may be high, and the blank value will also increase accordingly, resulting in a decrease in the accuracy of the kit.
9. Personal protective equipments are necessary in experiments for safety reasons. The stop solution in the kit is corrosive. Take care when using the reagent to avoid the risks. In case of accidental contact, please rinse with plenty of water and seek medical attention in time.
10. To avoid cross-contamination, use a new disposable pipette tips for each transfer. Please use disposable test tubes, pipette tips, plate sealers and clean plastic containers in the experiment.
11. Kit components from different batches or different sources cannot be used in combination.

For research use only, not for clinical diagnosis.

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