

Human IL-12 p70 ELISA Kit

Please read the manual carefully before use.

Cat. No. NE103

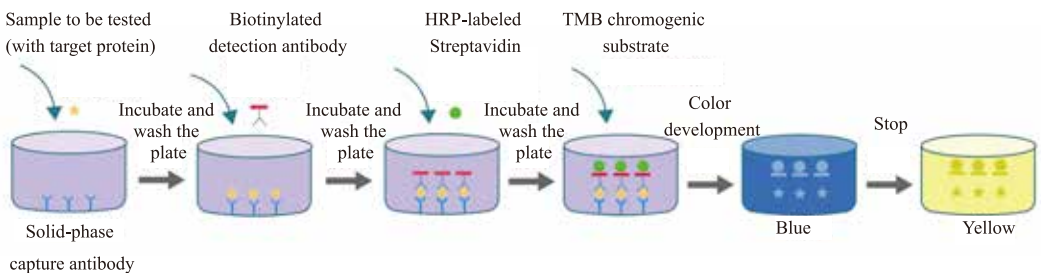
Storage: at 2~8°C in the dark for one year.



Description

Interleukin-12 (IL-12), also known as IL-12 p70, is a heterodimer composed of two subunits IL-12A (p35) and IL-12B (p40), with a molecular weight of 70-75 kD. IL-12 p70 is a secreted protein that is mainly produced and secreted by macrophages, dendritic cells, monocytes, neutrophils or other antigen-presenting cells in response to antigenic stimulation, it plays an important role in the maturation, activation, proliferation and immune regulation of immune cells. As a T cell stimulator, IL-12 p70 can effectively promote the proliferation and function of T cells, and participate in the differentiation of naïve T cells into Th1 cells. IL-12 p70 is a cytotoxic lymphocyte maturation factor. In the tumor microenvironment, IL-12 p70 stimulates activated T cells and natural killer cells to release toxic enzymes or secrete effector cytokines such as IFN- γ . These effector molecules are critical for tumor clearance.

This kit uses Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) to measure the amount of IL-12 p70 in human serum and plasma. The ELISA microplate in the kit is precoated with a high-affinity anti-human IL-12 p70 antibody. The standard or test sample is added to the microplate wells. After incubation, IL-12 p70 present in the sample will be bound specifically to the precoated antibody on the microplate wells. After washing, biotin-labeled anti-human IL-12 p70 detection antibody is added to the microplate wells. After re-incubation, the detection antibody will be bound specifically to IL-12 p70 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-12 p70 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-12 p70 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-12 p70 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

Serum, plasma, etc.



Kit Contents

| Component | NE103-01 | Storage |
|------------------------------------------------|--------------|----------------------|
| Human IL-12 p70 Antibody Precoated ELISA Plate | 96 T | at 2~8°C |
| Human IL-12 p70 Standard | 2 bottles | at 2~8°C |
| Standard & Sample Diluent | 15 ml/bottle | at 2~8°C |
| 100× Human IL-12 p70 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 washer or multi-channel pipette.

Sample Collection

1. 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.
2. 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. It is recommended to perform a pre-experiment to determine the dilution factor before the formal experiment.

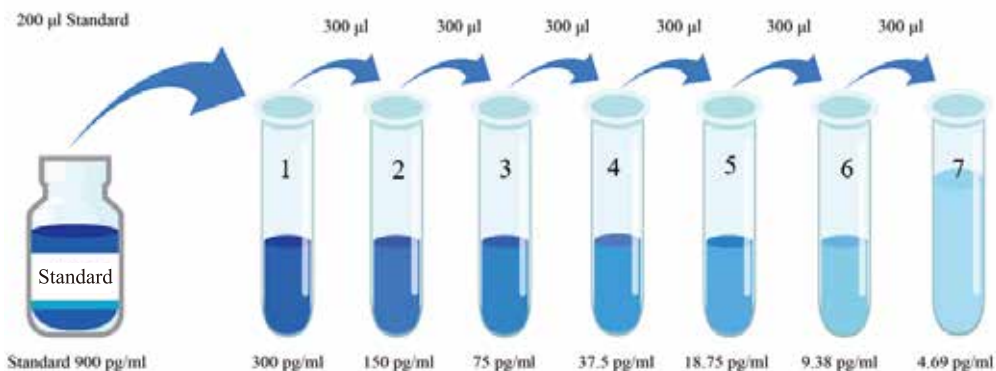
Working Solution Preparation

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-12 p70 Detection Antibody:** Spin and centrifuge the tube before use to collect the liquid on the tube wall at the bottom. Dilute 100× human IL-12 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.



- 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.
- 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.
- Dissolve Human IL-12 p70 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 900 pg/ml. Please use the redissolved standard within 30 minutes.
- Standard Gradient Dilution:** Dilute the redissolved standard 3 times with Standard & Sample Diluent, take 200 μ l of the reconstituted standard, add it to 400 μ l standard & sample diluent, mix well, record it as a tube 1, and the concentration of tube 1 at this time is 300 pg/ml; then perform a 2-fold gradient dilution according to the figure below, and add 300 μ l of standard & sample diluent to tubes 2-7 respectively; take 300 μ l 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 150 pg/ml. After mixing, take 300 μ l and add it to the tube 3. At this time, the concentration of the tube 3 is 75 pg/ml; and so on for serial dilution to tube 7 (4.69 pg/ml). 300 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.



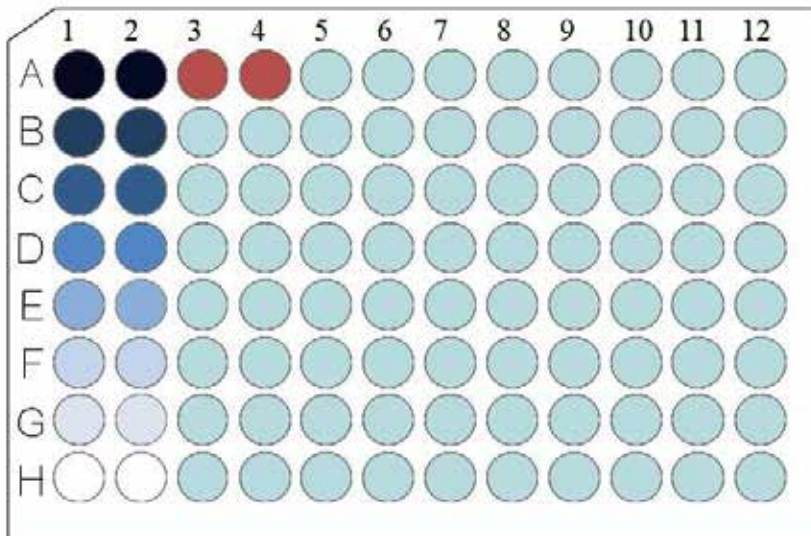
Experimental Protocol

- 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.
- Add the diluted standard and sample to the corresponding wells of the plate respectively, 100 μ l/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.
- Discard the liquid in the wells and wash wells the plate with 1× washing solution, 300 μ l/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.
- Add 1× Detection Antibody to each well, 100 μ l/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 300 pg/ml Standard
 B1/B2: 100 µl 150 pg/ml Standard
 C1/C2: 100 µl 75 pg/ml Standard
 D1/D2: 100 µl 37.5 pg/ml Standard
 E1/E2: 100 µl 18.75 pg/ml Standard
 F1/F2: 100 µl 9.38 pg/ml Standard
 G1/G2: 100 µl 4.69 pg/ml Standard
 H1/H2: 100 µl 0 pg/ml Standard (Standard & Sample Diluent)
 A3/A4: 100 µl Sample



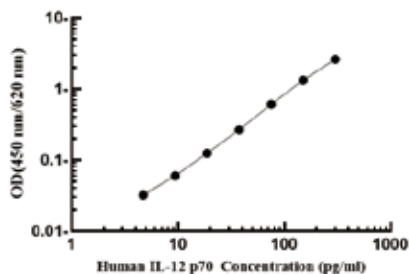
Result Analysis

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 |
|------------------|----------|-------|---------|-----------|
| 300 | 2.611 | 2.701 | 2.656 | 2.627 |
| 150 | 1.356 | 1.363 | 1.3595 | 1.3305 |
| 75 | 0.643 | 0.634 | 0.6385 | 0.6095 |
| 37.5 | 0.302 | 0.29 | 0.296 | 0.267 |
| 18.75 | 0.153 | 0.153 | 0.153 | 0.124 |
| 9.38 | 0.079 | 0.099 | 0.089 | 0.06 |
| 4.69 | 0.061 | 0.061 | 0.061 | 0.032 |
| 0 | 0.029 | 0.029 | 0.029 | 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 $(0.029+0.029)/2=0.029$, and the correction value is set as 0. The average OD value of the two duplicate wells of the 300 pg/ml standard is $(2.611+2.701)/2=2.656$, and the corrected value is $2.656-0.029=2.627$.

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) | 23.8 | 52.6 | 106.7 | 20.9 | 48.9 | 99.3 |
| Standard Deviation | 1.8 | 2.7 | 4.3 | 1.9 | 3.2 | 6.8 |
| Coefficient of Variation (%) | 7.6 | 5.1 | 4.0 | 9.1 | 6.5 | 6.8 |



Recovery Rate

Different concentrations of IL-12 p70 were added to the serum, plasma and cell culture supernate of 4 healthy people, and the samples without IL-12 p70 were used as the background to calculate the recovery rate.

| Sample | Average Recovery (%) | Range (%) |
|-----------------------|----------------------|-----------|
| Serum | 105 | 83~119 |
| EDTA Plasma | 107 | 89~120 |
| Sodium Citrate Plasma | 107 | 83~120 |
| Heparin Plasma | 89 | 86~101 |

Linearity

High concentrations of human IL-12 p70 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 (%) | 106 | 84 | 94 | 91 |
| | Range (%) | 103~108 | 81~89 | 86~98 | 89~92 |
| 1:4 | Average (%) | 108 | 82 | 112 | 93 |
| | Range (%) | 106~109 | 80~86 | 104~120 | 91~95 |
| 1:8 | Average (%) | 108 | 91 | 118 | 107 |
| | Range (%) | 106~109 | 86~96 | 110~116 | 101~116 |

Calibration

The standard of this kit is high-purity recombinant human IL-12 p70 calibrated by TransGen Biotech.

Sensitivity

The lowest detectable concentration of human IL-12 p70 was 2 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-12 p70 in 30 healthy human serum samples was detected by this kit, and the detection values of 30 samples were all lower than 4.69 pg/ml.

Specificity

This kit recognizes native and recombinant human IL-12 p70. Using recombinant human TNF- α , GM-CSF, IL-2, IL-23, IL-12B, IL-6, IL-10, FGF basic, IFN- γ 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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