Version 12-07/24

User's Manual

Human IL-12 (p70) ELISA Kit

REF **DEIA5130**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This kit is designed for quantitative detection of Human IL-12(p70) in sera, plasma, tissue or cell culture supernates.

General Description

Interleukin 12 (IL-12) is an interleukin that is naturally produced by dendritic cells, macrophages, neutrophils, helper T cells and human B-lymphoblastoid cells (NC-37) in response to antigenic stimulation. IL-12 belongs to the family of interleukin-12. IL-12 family is unique in comprising the only heterodimeric cytokines, which includes IL-12, IL-23, IL-27 and IL-35. Despite sharing many structural features and molecular partners, they mediate surprisingly diverse functional effects.

IL-12 is involved in the differentiation of naive T cells into Th1 cells. It is known as a T cell-stimulating factor, which can stimulate the growth and function of T cells. It stimulates the production of interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α) from T cells and natural killer (NK) cells, and reduces IL-4 mediated suppression of IFN-γ. T cells that produce IL-12 have a coreceptor, CD30, which is associated with IL-12 activity.

IL-12 plays an important role in the activities of natural killer cells and T lymphocytes. IL-12 mediates enhancement of the cytotoxic activity of NK cells and CD8+ cytotoxic T lymphocytes. There also seems to be a link between IL-2 and the signal transduction of IL-12 in NK cells. IL-2 stimulates the expression of two IL-12 receptors, IL-12R-β1 and IL-12R-β2, maintaining the expression of a critical protein involved in IL-12 signaling in NK cells. Enhanced functional response is demonstrated by IFN-γ production and killing of target cells.

Principles of Testing

The kit uses a double antibody sandwich ELISA method. Anti-human IL-12p70 monoclonal antibody is coated on the ELISA plate. Samples, standards and biotinylated anti-human IL-12p70 antibodies are added at the same time. IL-12p70 in the sample and standard will bind to the biotinylated anti-human antibody added to the well and the monoclonal antibody coated on the ELISA plate to form an immune complex. Avidin labeled with horseradish peroxidase is added. Avidin specifically binds to biotin. A colorimetric substrate (developer) is added. If there is IL-12p70 in the reaction well, horseradish peroxidase will turn the colorless developer blue. Add stop solution to turn yellow. Measure the OD value at 450 nm. There is a positive correlation between the IL-12p70 concentration and the OD450 value. The IL-12p70 concentration in the sample can be calculated by drawing a standard curve.

Reagents And Materials Provided

1. Human IL-12p70 Standard, 2 vials, 2-8°C
2. Human IL-12p70 Microplate, 8×12, 2-8°C
3. Human IL-12p70 100× Biotin-Antibody, 2×30 µL, 2-8°C
4. 100× HRP-Streptavidin, 2×60 µL, 2-8°C

5. Assay Diluent, 2×25 mL, 2-8°C
6. 20× Wash Buffer Concentrate, 30 mL, 2-8°C
7. Substrate Solution, 12 mL, 2-8°C(Avoid Direct Light)
8. Stop Solution, 12 mL, 2-8°C
9. Plate sealer, 2

Materials Required But Not Supplied

1. Microplate reader (Detection wavelength 450 nm, calibration wavelength 570 nm or 630 nm)
2. Precision single (0.5-10μL, 5-50μL, 20-200μL, 200-1000μL) and multi-channel pipette with disposable tips(calibration is required before use.)
3. 37°C incubator
4. Deionized or distilled water

Storage

2-8°C

Specimen Collection And Preparation

Sample collection:

1. The test tube for collecting blood should be a disposable non-pyrogenic test tube.
2. EDTA is recommended as the plasma anticoagulant. Hemolytic and hyperlipidemia specimens should be avoided.
3. The specimen should be clear and transparent. Suspended matter should be removed by centrifugation.
4. If the specimen is not tested in time after collection, it should be divided into a single-use amount and frozen in a -20°C/-80°C refrigerator to avoid repeated freezing and thawing.
5. According to the actual situation of the specimen, appropriate dilution can be made. (It is recommended to do a preliminary experiment to determine the dilution multiple).

Note: After the serum or plasma sample is frozen, the aggregated protein will cover the epitope of the antigen. It is recommended to dilute the serum or plasma sample with a reagent diluent at 1:2 (50 μL of sample, plus 50 μL of Assay Diluent) and then test. When calculating the sample content, multiply the dilution multiple (×2).

Serum sample collection:

Whole blood samples are placed at room temperature for 2 hours or at 2-8°C overnight, and then centrifuged at 2-8°C 1000g for 15 minutes. The supernatant can be tested immediately or stored in a -20°C/-80°C refrigerator to avoid repeated freezing and thawing.

Plasma sample collection:

Anticoagulants are recommended. Centrifuge samples for 15 minutes at 1000×g 2-8°C within 30 minutes after collection. Collect the supernatant to detect immediately. Or you can aliquot the supernatant and store it at -20°C or -80°C for future' s assay.

Cell supernatant collection:

Centrifuge the cell culture medium at 2-8°C to obtain the supernatant. The supernatant can be used for experiments immediately or stored in a -20°C/-80°C refrigerator to avoid repeated freezing and thawing.

Tissue samples:

1. Rinse the tissue with pre-cooled saline or PBS (0.15M, pH7.2) to remove blood. Wipe dry with filter paper, remove attached connective tissue, and weigh;
2. Use ophthalmic surgical scissors to chop up the tissue as quickly as possible. Add saline or PBS (0.15M, pH7.2), the concentration is recommended to be 0.1-1 g/mL (tissue wet weight 1:10-1:1);
3. Homogenization: There are many ways

   a. Manual homogenization: Put the chopped tissue into a pre-cooled mortar and grind it thoroughly to homogenize the tissue. You can also place the cut tissue directly in liquid nitrogen and then grind it in a mortar.

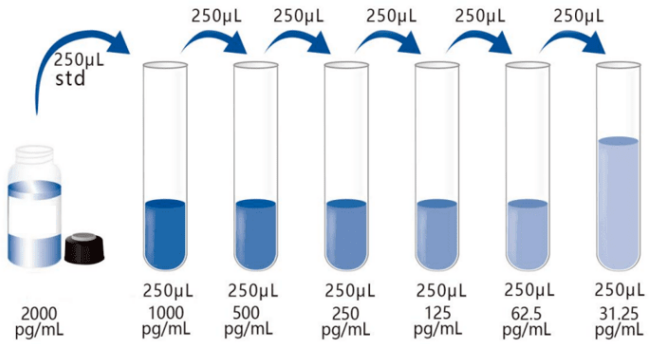
   b. Machine homogenization: Use a tissue crusher at 10000-15000 rpm to grind up and down to make tissue homogenate. It can also be prepared with an internal cutting tissue homogenizer (homogenization time 10 seconds/time, interval 30 seconds, continuous 3-5 times). Skin, muscle tissue, etc. can extend the homogenization time.

   c. Some tissues, such as liver, kidney, and brain tissue fluid, can be prepared by ultrasonic pulverization to prepare tissue homogenate.

   Note: To prevent protein degradation, all the above operations need to be performed on ice.
4. Centrifuge the prepared tissue homogenate at 2-8°C, 10000 rpm, for 10 minutes. Discard the precipitate and keep the supernatant.
5. Divide the tissue homogenate supernatant and store it at -20°C/-80°C for later use. Thaw and mix thoroughly before use.
6. Take an appropriate amount of supernatant for ELISA determination according to experimental needs. The dilution multiple of the test sample needs to be explored, and different multiples can be set for preliminary experiments. It is recommended to use Assay Diluent in the kit.

Reagent Preparation

1. It is recommended to take the kit out of the refrigerator 20 minutes in advance to equilibrate to room temperature.
2. Dilute 20× Wash Buffer Concentrate with deionized water to 1× Wash Buffer, and put the unused amount back into the refrigerator.
3. Standard: Add Assay Diluent to the lyophilized Human IL-12p70 Standard and dilute to 2000.0 pg/mL. Vortex to mix and let stand for 15 minutes. After it is fully dissolved, dilute it 2 times. The standard curve concentration is: 2000, 1000, 500, 250, 125, 62.5, 31.25, 0 pg/mL.



Note: If the reconstituted standard stock solution (2000 pg/mL) is not used up, please divide it into smaller pieces and store it in a refrigerator below -20°C. It can be stored for two months. Please discard the diluted standard.

4. 1× Biotin-Antibody: According to the amount required for the test, dilute Human IL-12p70 100× Biotin-Antibody 100 times with Assay Diluent to prepare 1× Biotin-Antibody. Prepare 30 minutes before use and only for the day.
5. 1× HRP-Streptavidin: Dilute 100× HRP-Streptavidin 100 times with Assay Diluent according to the amount required for the test to prepare 1× HRP-Streptavidin. Prepare 30 minutes before use and only for the day of use.

Assay Procedure

Washing Note:

1. Manual: Add 300 µL of 1×Wash Buffer to each well. Let stand for 10 seconds and shake off all the liquid in the well. Pat dry on absorbent paper. Wash the plate 5 times.
2. Automatic: Add 300 µL of 1×Wash Buffer to each well. The interval between injection and aspiration is 20-30 seconds.

Procedure:

1. Take out the required strips from the sealed bag that has been equilibrated to room temperature. Put the unused strips and desiccant back into the aluminum foil bag and seal at 2-8°C.
2. Set up blank wells. (If using dual wavelength plate reader, blank wells can be omitted).
3. Prepare samples, standards and 1× Biotin-Antibody in advance.
4. Add specimens or different concentrations of standards (0 pg/mL well plus reagent diluent) to the corresponding wells (100 µL/well). Then add 50 µL of 1× Biotin-Antibody working solution to the sample and standard wells, and seal the reaction wells with sealing tape.
5. Incubate at room temperature (20-25°C) for 120 minutes, using a micro-oscillator (frequency 300rpm).
6. Prepare 1× HRP-Streptavidin 30 minutes in advance. Place at room temperature away from light.
7. Wash the plate 5 times.
8. Add 1× HRP-Streptavidin (100 µL/well) to all wells except the blank well. Seal the wells with sealing tape.
9. Incubate at room temperature (20-25°C) for 60 minutes using a microvibrator (frequency 300rpm).
10. Wash the plate 5 times.

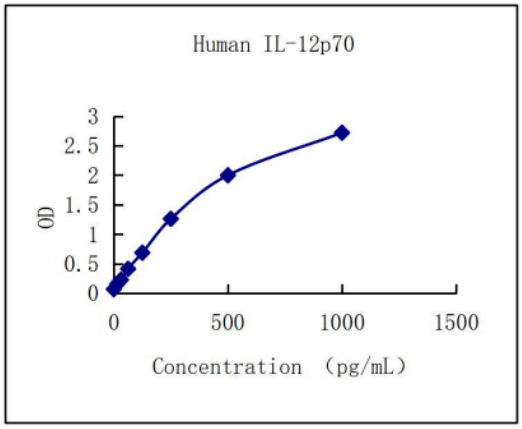
11. Add 100 µL/well of Substrate Solution (including blank wells) and incubate at room temperature (20-25°C) for 15 minutes in the dark.
12. Add 100 µL/well of Stop Solution (including blank wells), mix well, and immediately measure the OD450 value with an ELISA reader. (The ELISA reader is set to dual wavelength, with a detection wavelength of 450nm and a reference wavelength of 570 nm or 630nm. If the ELISA reader can only be set to read at a single wavelength, the OD value of the blank well should be subtracted from the OD value of each standard and sample).

Calculation

1. Preparation of standard curve: With the concentration of the standard as the x-axis and the OD value as the y-axis, fit and select the best standard curve (it is recommended to use a quadratic fitting equation). Calculate the sample concentration according to the sample OD value.
2. $R^2 \geq 0.99$. The deviation between the calculated concentration and the theoretical concentration at each point of the standard curve within the quantitative range should be within 15%, and the deviation between the upper and lower limits of quantification should be within 20%.
3. If the sample OD value is higher than the upper limit of the standard curve, it should be appropriately diluted and retested. When calculating the concentration, multiply the dilution factor.
4. The sample concentration should be calculated using the standard curve of the experiment.

Typical Standard Curve

Standard	OD
1000	2.719
500	1.998
250	1.26
125	0.686
62.5	0.413
31.25	0.226
15.625	0.158
0	0.068



Precautions

1. Please store the kit at 2-8°C before use. Except for the reconstituted standard, other components cannot be frozen.
2. 100× Biotin-Antibody and 100× HRP-Streptavidin are small in size. Bumping and possible inversion during transportation will cause the liquid to stick to the tube wall or bottle cap. Therefore, please centrifuge it instantly before use to allow the liquid attached to the tube wall or bottle cap to settle to the bottom of the tube.

3. The concentrated washing solution taken out of the refrigerator may have crystals, which is a normal phenomenon. Heat it to 37°C to completely dissolve the crystals before preparing the washing solution.
4. If the standard needs to be used in batches, it should be divided into each amount after reconstitution and stored at -18°C or below to avoid repeated freezing and thawing.
5. Avoid mixing components of kits with different batches.
6. Pay attention to fully mixing the solution to ensure that the liquid added to the well is uniform.
7. It is recommended to repeat the standard and sample at least twice in the ELISA test.