Version 27-04/24

User's Manual

Human High sensitive Interleukin 12 p70 ELISA Kit

REF **DEIA-LL283**

Σ **96T**

RUO

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

HS-IL-12 p70 ELISA Kit allows for the in vitro quantitative determination of IL-12 p70 concentrations in serum, plasma, tissue homogenates and other biological fluids.

General Description

IL-12 (interleukin 12) was first described as natural killer stimulating factor in 1989. The heterodimeric cytokine IL-12 consists of a 35-kd light chain (p35 or IL-12A) and 40-kd heavy chain (p40 or IL-12B). The gene encoding p35 is located on chromosome 3 in human beings and on chromosome 6 in mice. The p35 protein contains 197 amino acids and has homology to other single-chain cytokines (eg, IL-6 and G-CSF). The IL-12 p40 gene is on the human chromosome 5 in the same area as IL-3, IL-5, and GM-CSF, and the mice gene is on chromosome 11. P40 has homology to the extracellular domain of members of hematopoietic cytokinereceptor family (eg, IL-6Rα).

IL-12 (interleukin 12) receptor, binding interleukin 12, is composed of two subunits, IL-12RB1 (also known as CD212) and IL12RB2. These IL-12 functions depend on its IL-12 and IL-12 receptor complex. IL-12 (interleukin 12) is a heterodimeric pro-inflammatory cytokine that induces the production of interferon-γ (IFN-γ), favours the differentiation of T helper 1 (TH1) cells and forms a link between innate resistance and adaptive immunity.

Dendritic cells (DCs) and phagocytes produce IL-12 in response to pathogens during infection. Production of IL-12 is dependent on differential mechanisms of regulation of expression of the genes encoding IL-12, patterns of Toll-like receptor (TLR) expression and cross-regulation between the different DC subsets, involving cytokines such as IL-10 and type I IFN.

Principles of Testing

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Capture antibody was precoated onto 96-well plates. And the biotin conjugated antibody was used as detection antibodies. The standards, test samples and biotin conjugated detection antibody were added to the wells subsequently, and washed with wash buffer. HRP-Streptavidin (HRP-SA) and Biotin Systerm (BS) was added and unbound conjugates were washed away with wash buffer. Then by twice signal amplification, TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. Read the O.D. absorbance at 450nm in a microplate reader, and then the concentration of target can be calculated.

Reagents And Materials Provided

Item	Specifications(96T)	Storage
ELISA Microplate(Dismountable)	8×12	2-8°C/-20°C
Lyophilized Standard	2vial	2-8°C/-20°C
Sample/Standard Dilution Buffer	10ml	2-8°C
Assay Dilution	5ml	2-8°C
Biotin-labeled Antibody	10ml	2-8°C(Avoid Direct Light)
HRP-Streptavidin (HRP-SA)	20ml	2-8°C(Avoid Direct Light)
Biotin Systerm (BS)	120ul	2-8°C
BS Dilution Buffer	10ml	2-8°C
TMB Substrate	10ml	2-8°C(Avoid Direct Light)
Stop Solution	10ml	2-8°C
Wash Buffer(25X)	60ml	2-8°C
Plate Sealer	5pieces	
Product Description	1copy	

Materials Required But Not Supplied

1. Microplate reader (wavelength:450nm)
2. 37°C incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Storage

2-8 °C for 6 months

Specimen Collection And Preparation

Serum:
Place whole blood sample at room temperature for 2 hours or put it at 2-8°C overnight and centrifugation for 20 minutes at approximately 1000×g, Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.

Plasma:
Collect plasma using EDTA-Na_2 or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2-8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis, high cholesterol samples.

Tissue Homogenates:
As hemolysis blood has relation to assay result, it is necessary to remove residual blood by washing tissue with pre-cooling PBS buffer (0.01M, pH=7.4). Mince tissue after weighing it and get it homogenized in PBS

(the volume depends on the weight of the tissue. Normal, 9mL PBS would be appropriate to 1 gram tissue pieces. Some protease inhibitors are recommended to add into the PBS) with a glass homogenizer on ice. To further break the cells, you can sonicate the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates are then centrifuged for 5 minutes at 5000×g to get the supernatant. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3mg.

Cell Culture Supernatant:
Centrifuge supernatant for 20 minutes at 1000×g at 2-8°C to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately.

Cell Culture Lysate:
Commercial RIPA kits are recommended to follow the instructions provided. Generally, 0.5ml RIPA lysis buffer would be appropriate to $2x10^6$cells, DNA must to be removed. The total protein concentration was determined by BCA kit and the total protein concentration of each pore sample should not exceed 0.3mg.

Other Biological Fluids:
Centrifuge samples for 20 minutes at 1000×g at 2-8°C. Collect supernatant and carry out the assay immediately.

Note:
Samples to be used within 5 days can be stored at 2-8°C, besides that, samples must be stored at -20°C (assay ≤1 month) or -80°C(assay≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles. The hemolytic samples are not suitable for this assay.

Sample Dilution:
The user should estimate the concentration of target protein in the test sample, and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided dilution buffer, and several trials may be necessary. The test sample must be well mixed with the dilution buffer. And also standard curves and sample should be making in pre-experiment. If samples with very high concentrations, dilute samples with PBS first and then dilute the samples with Sample Dilution.

Reagent Preparation

Bring all reagents and samples to room temperature for 20 minutes before use.

Wash Buffer:

If crystals have formed in the concentrate, you can warm it with 40°C water bath (Heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.

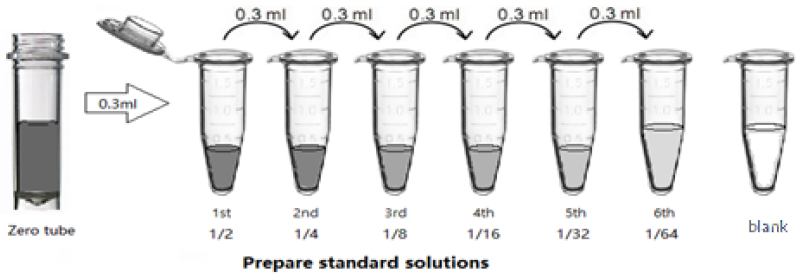
Dilute 30ml Concentrated Wash Buffer into 750ml Wash Buffer with deionized or distilled water. Put unused solution back at 2-8°C.

Standards:

Add 1 ml Sample Dilution Buffer into one Standard tube (labeled as zero tube), keep the tube at room temperature for 10 minutes and mix them thoroughly.

Note: If the standard tube concentration higher than the range of the kit, please dilute it and labeled as zero tube.

Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3ml of the Sample Dilution Buffer into each tube. Add 0.3ml of the above Standard solution (from zero tube) into 1st tube and mix them thoroughly. Transfer 0.3ml from 1st tube to 2nd tube and mix them thoroughly. Transfer 0.3ml from 2nd tube to 3rd tube and mix them thoroughly, and so on. Sample Dilution Buffer was used for the blank control.



Note: It is best to use Standard Solutions within 2 hours.

Preparation of BS Working Solution

Prepare it within 15 minutes before experiment.

1) Calculate required total volume of the working solution: 0.1ml/well × quantity of wells. (Allow 0.1-0.2ml more than the total volume.)
2) Dilute the BS with BS Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1ul of BS into 99ul of Poly-biotin Dilution Buffer.)

NOTE: If crystals have formed in the BS, you can warm it with water (temperature should not exceed 30°C) and mix it gently until the crystals have completely been dissolved.

Assay Procedure

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 minutes at 37°C. It is recommended to plot a standard curve for each test.

(1) Set standard, test samples, control (blank) wells on the pre-coated plate respectively, and then, records their positions. It is recommended to measure each standard and sample in duplicate.

(2)Add 50µL of Assay Diluent to each well.

(3)**Prepare Standards:** Aliquot 50ul of zero tube, 1stube, 2ndtube, 3rdtube, 4thtube, 5thtube, 6thtube and Sample Dilution Buffer (blank) into the sandard wells.

(4)**Add Samples:** Add 50ul of properly diluted sample into test sample wells.

(5)**Incubate:** Seal the plate with a cover and incubate at 37°C for 90 minutes.

(6)**Wash:** Remove the cover and discard the plate content, and wash plate 2 times with Wash Buffer. Do NOT let the wells dry completely at any time.

(7)**Biotin-labeled Antibody:** Add 100ul Biotin-labeled antibody working solution into above wells (standard, test sample and blank wells). Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37°C for 60 minutes.

(8)**Wash:** Remove the cover, and wash plate 2 times with Wash Buffer, and let the Wash Buffer stay in the wells for 1-2 minutes each time.

(9)**HRP-Streptavidin (HRP-SA)**: Add 100ul of HRP-SA into each well, cover the plate and incubate at 37°C for 30 minutes.

(10)**Wash:** Remove the cover and wash plate 3 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minutes each time.

(11)**BS Working Solution**: Add 100ul of BS Working solution into each well, cover the plate and incubate at room temperature for 15 minutes.

(12)**Wash**: Remove the cover and wash plate 3 times with Wash Buffer.

(13)**HRP-Streptavidin (HRP-SA)**: Add 100ul of HRP-SA into each well, cover the plate and incubate at 37°C for 30 minutes.

(14)**Wash**: Remove the cover and wash plate 3 times with Wash Buffer, and let the wash buffer stay in the wells for 1-2 minutes each time.

(15)**TMB Substrate**: Add 90ul TMB Substrate into each well, cover the plate and incubate at 37°C in dark within 10-20 minutes. (Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 minutes. You can terminate the reaction when apparent gradient appeared in standard wells.)

(16)**Stop**: Add 50ul Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.

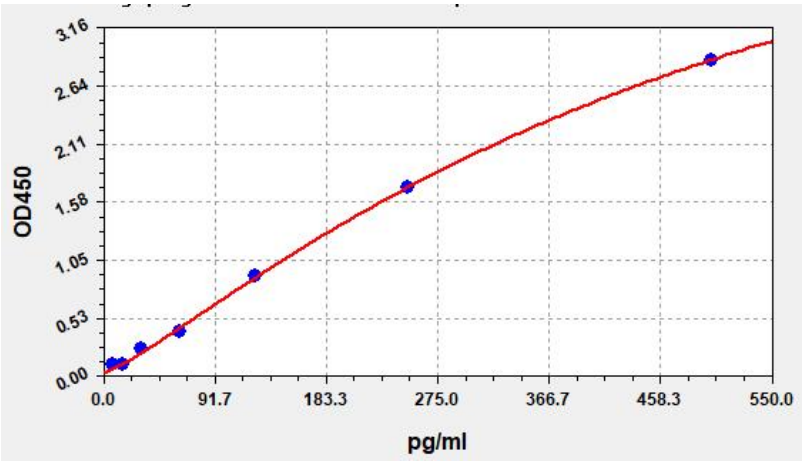
(17)**OD Measurement**: Read the O.D. absorbance at 450nm in Microplate Reader immediately after adding the stop solution.

Calculation

Regarding calculation, (the relative O.D.450) = (the O.D.450 of each well) – (the O.D.450 of blank well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The target concentration of the samples can be interpolated from the standard curve. It is recommended to use some professional software to do this calculation.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Typical Standard Curve



Precision

Intra-assay:CV<8%;

Inter-assay:CV<10%

Detection Range

7.8-500pg/ml

Sensitivity

< 4.6pg/ml

Specificity

This assay has high sensitivity and excellent specificity for detection of IL-12 p70. No significant crossreactivity or interference between IL-12 p70 and analogues were observed. Note: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between IL-12 p70 and all the analogues, therefore, cross reaction may still exist.

Precautions

1. To inspect the validity of experiment operation and the appropriateness of sample dilution proportion, pilot experiment using standards and a small number of samples is recommended.
2. After opening and before using, keep plate dry.
3. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
4. Storage TMB reagents avoid light.
5. Washing process is very important, not fully wash easily cause a false positive and high background.
6. Duplicate well assay is recommended for both standard and sample testing.
7. Don't let microplate dry at the assay, for dry plate will inactivate active components on plate.
8. Don't reuse tips and tubes to avoid cross contamination.
9. Avoid using the reagents from different batches together.
10. Lightly Seal the plate, if the well is sealed too tight, it is easy to produce steam, which will affect the test results.