



Product Information Sheet

Human IL-12(p70) ELISA Kit

Catalog No.	EK0421
Size	96T
Range	7.8pg/ml-500pg/ml
Sensitivity	< 2 pg/ml
Specificity	No detectable cross-reactivity with any other cytokine.
Storage	Store at 4°C for frequent use, at -20°C for infrequent use. Avoid multiple freeze-thaw cycles (Shipped with wet ice.)
Expiration	Four months at 4°C and eight months at -20°C.
Application	For quantitative detection of human IL-12(p70) in sera, plasma, body fluids, tissue lysates or cell culture supernates.

Principle

Human IL-12(p70) ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human IL-12(p70) specific-specific monoclonal antibodies were precoated onto 96-well plates. The human specific detection monoclonal antibodies were biotinylated. The test samples and biotinylated detection antibodies were added to the wells subsequently and then followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex was added and unbound conjugates were washed away with PBS or TBS buffer. HRP substrate TMB was 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 human IL-12(p70) amount of sample captured in plate.

Kit Components

1. Lyophilized recombinant human IL-12(p70) standard: 10ng/tube×2.
2. One 96-well plate precoated with anti- human IL-12(p70) antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human IL-12(p70) antibody : 130µl, dilution 1:100.
5. Antibody diluent buffer: 12ml.
6. Avidin-Biotin-Peroxidase Complex (ABC) : 130µl, dilution 1:100.
7. ABC diluent buffer: 12ml.
8. TMB color developing agent: 10ml.
9. TMB stop solution: 10ml.

Material Required But Not Provided

1. Microplate reader in standard size and Automated plate washer.
2. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
3. Clean tubes and Eppendorf tubes.
4. Washing buffer (neutral PBS or TBS).

Preparation of 0.01M **TBS**: Add 1.2g Tris, 8.5g NaCl; 450µl of purified acetic acid or 700µl of concentrated hydrochloric acid to 1000ml H₂O and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

Preparation of 0.01 M **PBS**: Add 8.5g sodium chloride, 1.4g Na₂HPO₄ and 0.2g NaH₂PO₄ to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

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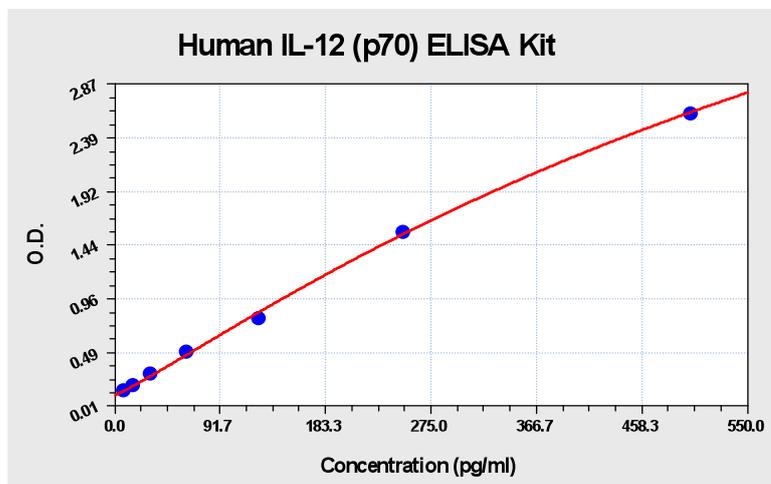
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Notice for Application of Kit

1. Before using Kit, spin tubes and bring down all components to bottom of tube.
2. Duplicate well assay was recommended for both standard and sample testing.
3. Don't let 96-well plate dry, dry plate will inactivate active components on plate.
4. In order to avoid marginal effect of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution will be pre-warmed in 37°C for 30 min before using.

Human IL-12(p70) ELISA Kit-1X96 Well Plate Image



Background

Interleukin (IL)-12 is a 70-KDa cytokine comprised of two disulfide-linked proteins (p35 and p40) and is essential for the initiation of effective immune response.ⁱ And the IL-12p70 is a heterodimer of p35 and p40 subunits; it is an important cytokine secreted by antigen-presenting cells in response to antigenic stimulation.ⁱⁱ Gene expression analysis of the IL-12 cytokine family subunits revealed that both strains induced high levels of p40 (protein chain communal to IL-12 p70 and IL-23) as well as p19, a subunit of IL-23. Conversely only ACT- 18HS19 infection induced consistent transcription of IL-12 p35, a subunit of IL-12 p70.ⁱⁱⁱ The standard product used in this kit is recombinant human IL-12 p70 with the molecular mass of 75 KDa.

Reference

1. Toubai T, Tanaka J, Ota S, Fukuhara T, Hashino S, Kondo T, Shono Y, Morioka M, Kawamura T, Masauzi N, Kakinoki Y, Kobayashi H, Kunieda Y, Kasai M, Kurosawa M, Asaka M, Imamura M. Effect of granulocyte colony-stimulating factor on IL-12 p40 production during chemotherapy for B-cell lineage non-Hodgkin's lymphoma patients. *Eur J Haematol.* Nov;77(5):403-9.2006.
2. Nakamura T, Kimura H, Kato M, Kurashige S, Wakamatsu K. A sensitive and reliable quantification method for mouse interleukin-12 p70 based on fluorometric sandwich ELISA (FS-ELISA). *Cell Biol Int.* Feb;31(2):173-9.2007
3. Spensieri F, Fedele G, Fazio C, Nasso M, Stefanelli P, Mastrantonio P, Ausiello CM. Bordetella pertussis inhibition of interleukin-12 (IL-12) p70 in human monocyte-derived dendritic cells blocks IL-12 p35 through adenylate cyclase toxin-dependent cyclic AMP induction. *Infect Immun.* May;74(5):2831-8,2006.

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