Product Information Sheet

Human IL-12(p40) ELISA Kit

Catalog No. EK0423

Size 96T

Range 31.2pg/ml-2000pg/ml

Sensitivity < 2 pg/ml

Specificity

Cross-reactivates with IL-12(p70), IL-23(p19/p40) < 5%.

Storage

Store at 4℃ for frequent use, at -20℃ for infrequent use.

Avoid multiple freeze-thaw cycles (Shipped with wet ice.)

Expiration

Four months at 4℃ and eight months at -20℃.

Application

For quantitative detection of human IL-12(p40) in sera, plasma, body fluids, tissue lysates or cell culture supernates.

To reorder contact us at:

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Principle

Human IL-12(p40) ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human IL-12(p40) specific-specific monoclonal antibodies were precoated onto 96-well plates. The human specific detection polyclonal 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(p40) amount of sample captured in plate.

Kit Components

1. Lyophilized recombinant human IL-12(p40) standard: 10ng/tube×2.
2. One 96-well plate precoated with anti- human IL-12(p40) antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human IL-12(p40) 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 H_2O 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 Na_2HPO_4 and 0.2g NaH_2PO_4 to 1000ml distilled water and adjust pH to 7.2-7.6. Finally, adjust the total volume to 1L.

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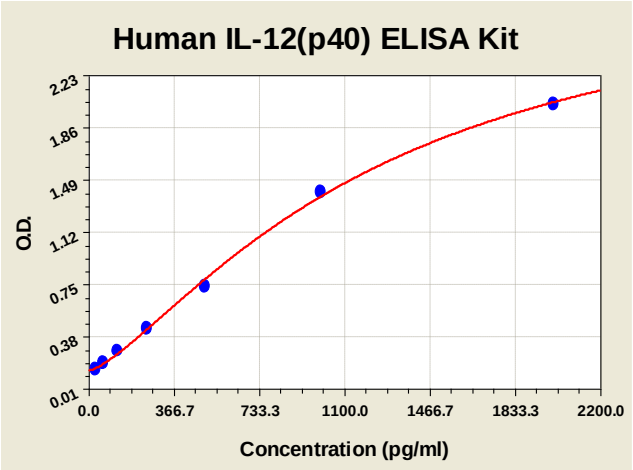
Notice for Application of Kit

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℃ for 30 min before using.

Human IL-12(p40) ELISA Kit-1X96 Well Plate Image



Background

Interleukin-12 (IL12; formerly NKSF, for natural killer cell stimulatory factor, or CLMF, for cytotoxic lymphocyte maturation factor) is a novel cytokine cloned from B-cell lines. IL-12 is a heterodimeric molecule composed of p35 and p40 subunits.[1] The larger 40-kDa subunit (p40) is a member of the cytokine receptor family, and the smaller 35-kDa subunit (p35) is related to IL6 and GCSF.[2] Both IL-12 p40(-/-) and p35(-/-) mice fail to produce IL-12 p70 heterodimer.[3] Interleukin (IL)-12 has been cloned on the basis of its ability to activate natural killer (NK) cells and promote the development of cytolytic T cells. With further understanding of its activities, IL-12 has emerged as an important cytokine, affecting both immune and hematologic functions. It has been shown to be necessary for the T cell independent induction of interferon (IFN)-gamma, critical for the initial suppression of bacterial and parasitic infection; for the development of a Th1 response, critical for effective host defense against intracellular pathogens; and for the activation of differentiated T lymphocytes of both CD4+ and CD8+ phenotype.[4] The subunits map to different chromosomes: p40 (IL12B) to 5q31-q33 and p35 (IL12A) to 3p12-3q13.2.[2] The standard product used in this kit is recombinant human IL-12(p40), which is a 40KDa glycoprotein consisting of 306 amino acids.

Reference

1. Cua, D. J.; Sherlock, J.; Chen, Y.; Murphy, C. A.; Joyce, B.; Seymour, B.; Lucian, L.; To, W.; Kwan, S.; Churakova, T.; Zurawski, S.; Wiekowski, M.; Lira, S. A.; Gorman, D.; Kastelein, R. A.; Sedgwick, J. D. Interleukin-23 rather than interleukin-12 is the critical cytokine for autoimmune inflammation of the brain. *Nature* 421: 744-748, 2003.
2. Sieburth, D.; Jabs, E. W.; Warrington, J. A.; Li, X.; Lasota, J.; LaForgia, S.; Kelleher, K.; Huebner, K.; Wasmuth, J. J.; Wolf, S. F. Assignment of genes encoding a unique cytokine (IL12) composed of two unrelated subunits to chromosomes 3 and 5. *Genomics* 14: 59-62, 1992.
3. Becher, B.; Durrell, B. G.; Noelle, R. J. Experimental autoimmune encephalitis and inflammation in the absence of interleukin-12. *J. Clin. Invest.* 110: 493-497, 2002.
4. Wolf, S. F.; Sieburth, D.; Sypek, J. Interleukin 12: a key modulator of immune function. *Stem Cells* 12: 154-168, 1994.

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