

Human IL-4 ELISA Kit

#Cat: NB-06-0909

Size: 96T

Range: 7.8pg/ml-500pg/ml

Sensitivity: < 1.5pg/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-4 in sera, plasma, body fluids, tissue lysates or cell culture supernates.

Principle:

Neo Biotech's human IL-4 ELISA Kit was based on standard sandwich enzyme-linked immune-sorbent assay technology. Human IL-4 specific-specific polyclonal 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-4 amount of sample captured in plate.

Kit Components:

1. Lyophilized recombinant human IL-4 standard: 10ng/tube×2.
2. One 96-well plate precoated with anti- human IL-4 antibody.
3. Sample diluent buffer: 30 ml
4. Biotinylated anti- human IL-4 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.
2. Automated plate washer.
3. Adjustable pipettes and pipette tips. Multichannel pipettes are recommended in the condition of large amount of samples in the detection.
4. Clean tubes and Eppendorf tubes.
5. 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.

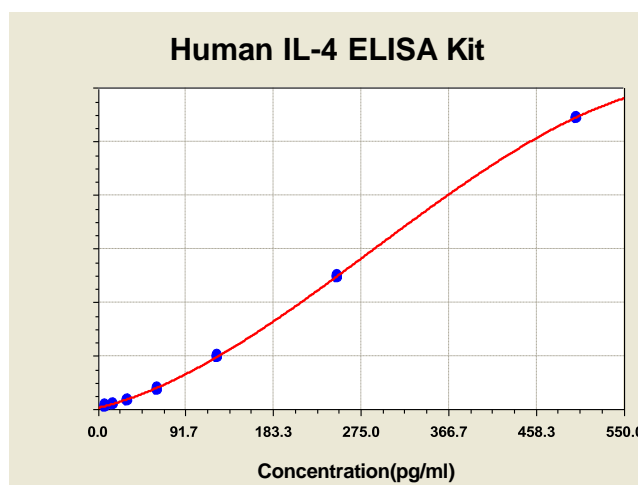
Notice for Application of Kit:

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.

Typical Data Obtained from Human IL-4:

(TMB reaction incubate at 37°C for 25 min)

Concentration(pg/ml)	0.0	15.6	31.2	62.5	125	250	500	1000
O.D	0.089	0.130	0.157	0.209	0.379	0.772	1.537	2.465



Background

Interleukin-4 (IL-4), also known as a B-cell stimulatory factor1 (BSF1), is an immunomodulatory cytokine, which can inhibit the growth of tumour cells.¹ The human cDNA contains a single open reading frame encoding a protein of 153 amino acids, including a putative signal peptide. IL-4 may act as an autocrine growth factor in pancreatic cancer cells and also give rise to the possibility that cancer-derived IL-4 may suppress cancer-directed immunosurveillance in vivo in addition to its growth-promoting effects, thereby facilitating pancreatic tumor growth and metastasis.¹ The mouse and human genes and their protein products show structural and functional similarities. The human IL-4 gene, which occurs as a single copy in the haploid genome, is mapped on chromosome 5.² The standard product used in this kit is recombinant human IL-4, consisting of 130 amino acids with the molecular mass of 14KDa.

Reference

1. Prokopchuk, O.; Liu, Y.; Henne-Bruns, D.; Kornmann, M. Interleukin-4 enhances proliferation of human pancreatic cancer cells: evidence for autocrine and paracrine actions. *Br J Cancer*. 2005 Mar 14;92(5):921-8.
2. Arai, N.; Nomura, D.; Villaret, D.; DeWaal Malefijt, R.; Seiki, M.; Yoshida, M.; Minoshima, S.; Fukuyama, R.; Maekawa, M.; Kudoh, J.; Shimizu, N.; Yokota, K.; Abe, E.; Yokota, T.; Takebe, Y.; Arai, K. Complete nucleotide sequence of the chromosomal gene for human IL-4 and its expression. *J. Immun.* 142: 274-282, 1989.

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