Human Interleukin 12, IL12 ELISA Kit

Cat. No.: DEIA117
Lot. No.: (See product label)
Pkg.Size: 10 Plates

INTENDED USE

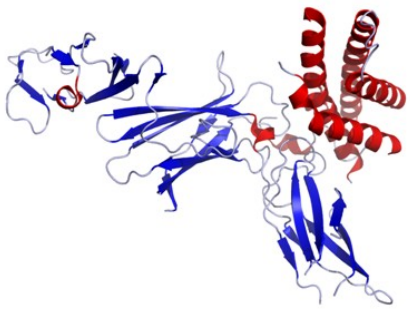
Human IL-12 ELISA development kit contains the key components required for the quantitative measurement of natural and/or recombinant IL-12 in a sandwich ELISA format within the range of 32–3,000 pg/ml. Using the ELISA protocol described below, this kit provides sufficient reagents to assay IL -12 in approximately 1,000 ELISA plate wells.

DESCRIPTION

Inerleukin-12 (IL-12), as a potent factor, plays an important role in the networks of immune system. It is a multifunctional cytokine, the properties of which bridge innate and adaptive immunity, acting as a key regulator of cell-mediated immune responses through the induction of T helper 1differentiation. By promoting IFN-γ production, proliferation, and cytolytic activity of natural killer and T cells, IL-12 induces cellular immunity.

RECONSTITUTION & STORAGE

Capture Antibody: 100 µg of antigen-affinity purified goat anti-IL-12. Centrifuge vial prior to opening. Reconstitute in 1.0 ml sterile water for a concentration of 100 µg/ml. Following reconstitution the Capture antibodies may be stored at 2-8℃ for up to 6 months. For long term storage it is recommended to aliquot into working volumes and store at -70℃ in a manual defrost freezer.
Detection Antibody: 5 µg of biotinylated antigen-affinity purified goat anti-IL-12. Centrifuge vial prior to opening. Reconstitute in 0.25 ml sterile water for a concentration of 100 µg/ml. Following reconstitution the Detection antibodies may be stored at 2-8℃ for up to 6 months. For long term storage it is recommended to aliquot into working volumes and store at -70℃ in a manual defrost freezer.
Human* IL-12 *Standard: 1 µg of recombinant IL-12. Centrifuge vial prior to opening. Reconstitute in 1.0 ml sterile water for a concentration of 1 µg/ml. The Standard may be stored at 2-8℃ for one month or aliquoted and stored at -70℃ for up to three months in a manual defrost freezer.
UltraAvidin-HRP Conjugate: 40 µl vial. Upon receipt, UltraAvidin-HRP conjugate should be stored at 2-8℃, **DO NOT FREEZE.**
TMB Liquid Substrate: Aspirate and wash plate 4 times. Add 100 µl of TMB HRPO Microwell Substrate Standard Kinetic One Component "Ready Use" to each well. Incubate at room temperature and monitorcolor development with an ELISA plate reader at 450 nm with wavelength correction set at 540 nm or 570 nm. The reaction may be stopped after 15 – 20 minutes by adding 100 µl of 2 M sulfuric acid to each well.



Crystal structure of human IL-12

ANALYTE GENE INFORMATION

Gene Name: IL12A interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35) [*Homo sapiens*]
Official Symbol: IL12A
Synonyms: interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35); P35; CLMF; NFSK; NKSF1; IL-12A; natural killer cell stimulatory factor 1, 35 kD subunit; cytotoxic lymphocyte maturation factor 1, p35; interleukin 12 p35;IL-12 subunit p35;NF cell stimulatory factor chain 1; interleukin-12 alpha chain; IL12A_HUMAN; Interleukin-12 subunit alpha [Precursor];IL-12 subunit p35;Cytotoxic lymphocyte maturation factor 35 kDa subunit;CLMF p35; IL12A; OTTHUMP00000160820
GeneID: 3592
mRNA Refseq: NM_000882
Protein Refseq: NP_000873
MIM: 161560
UniProt ID: P29459
Chromosome Location: 3q25.33-q26
Function: Contributes to cytokine activity; interleukin-12 beta subunit binding; interleukin-12 receptor binding; interleukin-27 binding; protein binding; protein heterodimerization activity.

RECOMMENDED MATERIALS

- ELISA microplates;
- BSA;
- Stop Solution 2 M Sulfuric Acid;
- Dulbecco's PBS [10x]

RECOMMENDED SOLUTIONS

PBS: Dilute 10xPBS to 1xPBS, pH 7.2 in sterile water.
Wash Buffer: 0.05% Tween-20 in PBS.
Block Buffer: 1.0% BSA in PBS.
Diluent: 1.0% BSA in PBS.

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PLATE PREPARATION

1. Dilute to 2.0 µg/ml of capture antibody and immediately add 100 µl to each ELISA plate well. Seal the plate and incubate overnight at room temperature.
2. Aspirate the wells to remove liquid and wash the plate 4 times using 300µl of wash buffer per well.
3. After the last wash invert plate to remove residual buffer and blot on paper towel.
4. Add 300µl block buffer to each well. Incubate for at least 1 hour at room temperature.
5. Aspirate and wash plate 4 times.

ELISA PROTOCOL

Standard/Sample: Dilute standard from 2,000 pg/ml to zero in diluent. Immediately add 100 µl of standard or sample to each well in duplicate and incubate at room temperature for at least 2 hours.
Detection: Aspirate and wash plate 4 times. Dilute a portion of detection antibody in diluent to a concentration of 50 ng/ml. Add 100 µl per well and incubate at room temperature for 2 hours.
UltraAvidin-HRP Conjugate: Aspirate and wash plate 4 times. Dilute 3.0 µl of avidin-HRP conjugate 1:5,000 (this dilution factor may require some optimization) in diluent for a total volume of 15 ml. Add 100µl per well and incubate at room temperature for 30 minutes.
TMB Liquid Substrate: Add 100 µl of TMB HRPO Microwell Substrate Standard Kinetic One Component "Ready Use" to each well. Incubate at room temperature for 20-30 minutes and monitor color development with an ELISA plate reader at 450 nm with wavelength correction set at 540 nm or 570 nm. Avoid placing plates in direct light.
Stop Solution: The reaction may be stopped after 15 – 20 minutes by adding 100 µl of 2 M sulfuric acid to each well. All eye, hand, face, and clothing protection should be used when working with sulfuric acid.

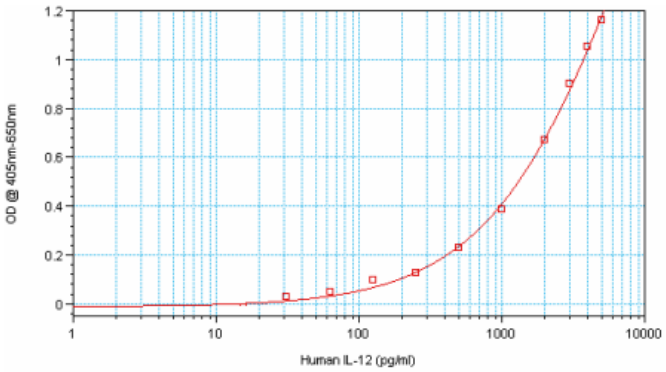
REFENRENCES

1. Mells GF, *et al*. Nat Genet, 2011 Mar 13. PMID 21399635.
2. Kumar R, *et al*. Cell Immunol, 2011. PMID 21145044.
3. Vasakova M, *et al*. Sarcoidosis Vasc Diffuse Lung Dis, 2010 Jul. PMID 21086908.

CROSS REACTIVITY

When tested at 50 ng/ml the following antigen exhibited less than 5% cross reactivity: Human IFNγ, IL-10, IL-16 (121aa), IL-16 (130aa), IL-17A, B, D, E and F. Mouse IL-12, IFNγ, IL-10 and IL-12p40. Rat IFNγ.

TYPICAL STANDARD CURVE



The standard curve is provided as an example only. A standard curve should be prepared with each assay.

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