Human Interleukin 8, IL8 ELISA Kit

Cat. No.: DEIA124
Lot. No.: (See product label)
Pkg.Size: 10 Plates

PDB rendering based on 1IL8.

INTENDED USE

Human IL-8 ELISA development kit contains the key components required for the quantitative measurement of natural and/or recombinant IL-8 in a sandwich ELISA format within the range of 8–2,000 pg/ml. Using the ELISA protocol described below, this kit provides sufficient reagents to assay IL-8 in approximately 1,000 ELISA plate wells.

DESCRIPTION

Interleukin-8 (IL-8) is a chemokine, a member of the cytokine family that displays chemotactic activity for specific types of leukocytes. It is a proinflammatory CXC chemokine that can signal through the CXCR1 and CXCR2 receptors. Many cell types, including monocyte/macrophages, T cells, neutrophils, fibroblasts, endothelial cells, keratinocytes, hepatocytes, chondrocytes, and various tumor cell lines, can produce CXCL8/IL-8 in response to a wide variety of pro-inflammatory stimuli such as exposure to IL-1, TNF, LPS, and viruses.

RECONSTITUTION & STORAGE

Capture Antibody: 50 µg of antigen-affinity purified goat anti-IL-8. Centrifuge vial prior to opening. Reconstitute in 0.5 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: 25 µg of biotinylated antigen-affinity purified goat anti-IL-8. 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-8 Standard: 1 µg of recombinant IL-8. 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

ANALYTE GENE INFORMATION

Gene Name: IL8 interleukin 8 [*Homo sapiens*]
Official Symbol: IL8
Synonyms: NAF; GCP1; LECT; LUCT; NAP1; CXCL8; GCP-1; LYNAP; MDNCF; MONAP; NAP-1; IL8 ; interleukin-8; emoctakin; OTTHUMP00000199825; C-X-C motif chemokine 8; T cell chemotactic factor; T-cell chemotactic factor; neutrophil-activating peptide 1; chemokine (C-X-C motif) ligand 8; beta-thromboglobulin-like protein; granulocyte chemotactic protein 1; monocyte-derived neutrophil chemotactic factor; monocyte-derived neutrophil-activating peptide; small inducible cytokine subfamily B, member 8; lymphocyte-derived neutrophil-activating factor.
GeneID: 3576
mRNA Refseq: NM_000584
Protein Refseq: NP_000575
MIM: 146930
UniProt ID: P10145
Chromosome Location: 4q13-q21
Function: chemokine activity; interleukin-8 receptor binding; protein binding

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.

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.

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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.

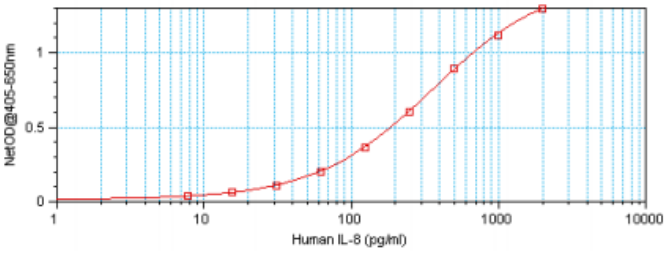
REFENRENCES

1. Haraldsen G et al. (1998) J Exp Med. 188:1751.

CROSS REACTIVITY

When tested at 50 ng/ml the following antigen exhibited less than 5% cross reactivity: Human BCA-1, BRAK, CXCL16, ENA-78, Fractalkine, GCP-2, GRO, GRO-β, GRO-γ, I-TAC, IP-10, Lymphotactin, MCP-1, MIG, NAP-2, PF-4, SDF-1α and SDF-1β.

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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