Human Interleukin 8, IL8 ELISA Kit

Prod. No.: DEIA165
Pkg.Size: 96T

INTENDED USE

This assay is a sandwich Enzyme Linked-Immuno-Sorbent Assay (ELISA). It is developed for quantitative measurement of Human IL8 in serum, plasma and other biological fluids.

PRINCIPLE OF THE TEST

An antibody specific for Human IL8 is coated onto the wells of the microtiter plate. Samples and standards of Human IL8 are pipetted into the wells for binding to the coated antibody. After washing procedure to remove unbound compounds, an enzyme-linked antibody specific for Human IL8 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of Human IL8 bound in the initial step. The color development is stopped and the intensity of the color is measured. The magnitude of the absorbance for this developed color is proportional to the amount of Human IL8.

SPECIFICITY&SENSITIVITY

Specificity: This assay recognizes Human IL8. No significant cross-reactivity or interference was observed.
Detection Rage: 31.25-2,000 pg/ml.
Detection Limit: The minimum detectable dose of Human IL8 is typically less than 7 pg/ml.

REAGENTS AND MATERIALS PROVIDED

Human IL8 Microplate: polystyrene microplate coated with a monoclonal antibody against Human IL8;
Standard (freeze dried): 2,000 pg/ml, 2 vials;
Standard or Sample Diluent: 16ml, 1 vials;
Biotin-antibody (100×): 60ul, 2 vials;
Biotin-antibody Diluent: 16ml, 1 vials;
HRP-avidin (100×): 60ul, 2 vials;
HRP-avidin Diluent: 16ml, 1 vials;
TMB Substrate: 12ml, 1 vials;
TMB Stop Solution: 12ml, 1 vials;
Wash Buffer (20×): 25ml, 1 vials;
Microtiter plate sealers

ANALYTE GENE INFORMATION

Gene Name: IL8 interleukin 8 *[Homo sapiens]*
Official Symbol: IL8
Synonyms: IL8; interleukin 8; CXCL8; GCP-1; GCP1; LECT; LUCT; LYNAP; MDNCF; MONAP; NAF; NAP-1; NAP1; K60; T cell chemotactic factor; beta-thromboglobulin-like protein; chemokine (C-X-C motif) ligand 8; lymphocyte-derived neutrophil-activating factor; emoctakin; granulocyte chemotactic protein 1; monocyte-derived neutrophil chemotactic factor; neutrophil-activating peptide 1; T-cell chemotactic factor; small inducible cytokine subfamily B, member 8; b-ENAP; Monocyte-derived neutrophil chemotactic factor; Monocyte-derived neutrophil-activating peptide; Neutrophil-activating protein 1
GeneID: 3576
mRNA Refseq: NM_000584
Protein Refseq: NP_000575
MIM: 146930
UniProt ID: P10145
Chromosome Location: 4q13-q21
Pathway: Cytokine-cytokine receptor interaction; Pathways in cancer; Toll-like receptor signaling pathway; Bladder cancer; Chemokine signaling pathway; Epithelial cell signaling in Helicobacter pylori infection; RIG-I-like receptor signaling pathway; Signaling by GPCR
Function: interleukin-4 receptor activity; cytokine activity; chemokine activity; protein binding

MATERIALS REQUIRED BUT NOT SUPPLIED

1. Validated microplate reader.
2. Eppendorf Tubes for dilution for samples and standards.
3. Deionized or distilled water.
4. Validated adjustable micropipettes, single and multi-channel.
5. Automatic microtiter plate washer or manual vacuum aspiration equipment.
6. 37°C incubator.

STORAGE

Unopened Kit: Store at 2 - 8°C. Do not use past kit expiration date.
Opened/Reconstituted Reagents: Standard or Sample Diluent; Biotin-antibody Diluent; HRP-avidin Diluent; TMB Substrate; Wash Buffer; TMB Stop Solution. The above mentioned reagents should be stored for up to 1 month at 2 - 8°C.
Microplate Wells: Return unused wells to the foil pouch containing the desiccant pack, reseal along entire edge of zip-seal. May be stored for up to 1 month at 2 - 8°C.

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RECONSTITUTION AND STORAGE

Sample: Centrifuge the serum, plasma or cell culture supernatant samples for 10 minutes at 1,000×g. Remove particulates and assay immediately or aliquot and store samples at -20° C or -80° C. Avoid repeated freeze-thaw cycles.
Standard: Centrifuge the standard vial at 6,000-10,000rpm for 30s. Reconstitute the Standard with 1.0 ml of standard or Sample Diluent. The undiluted standard serves as the high standard (2,000 pg/ml). The Sample Diluent serves as the zero standard (0 pg/ml). Prepare fresh for each assay. Prepare within 2 hours of use.
Biotin-antibody: Centrifuge the vial before opening. Dilute to the working concentration using Biotin-antibody Diluent (1:100), respectively. Prepare within 1 hour of use.
HRP-avidin: Centrifuge the vial before opening. Dilute to the working concentration using HRP-avidin Diluent(1:100), respectively. Prepare within 1 hour of use.
Wash Buffer: If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20× Wash Buffer Concentrate into deionized or distilled water.

PRECAUTIONS

1. The kit should be equilibrated to room temperature (20-23° C) before opening any vials and starting the assay. It is highly recommended that the solutions be used as soon as possible after rehydration.
2. When mixing or reconstituting protein solutions, always avoid foaming.
3. Do not mix or substitute reagents with those from other lots or sources.
4. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
5. Crystals could appear in the 20X wash solution due to high salt concentration in the stock solutions. Crystals are readily dissolved at room temperature or at 37°C before dilution of the buffer solutions.
6. Keep TMB Substrate protected from light.
7. The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

ELISA PROTOCOL

1. Prepare all reagents, working standards, and samples as directed in the previous sections. Dilute original density Standard as follow: Set up 7 points of diluted standard such as 2,000 pg/ml, 1,000 pg/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml and 31.25 pg/ml. The last EP tubes with Sample Diluent is the blank as 0 pg/ml.

2. Add 100µl of Standard, Control or Sample per well. Cover with the Microtiter plate sealers. Incubate for 1.5 hours at 37°C.

3. Aspirate each well and wash, Wash by filling each well with 1 x Wash Buffer using a squirt bottle, multi-channel pipette, manifold dispenser or autowasher. Complete removal of liquid at each step is essential to good performance. Repeating the process twice for a total of four washes. After the last wash, remove any remaining 1× Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100µl of Biotin-antibody working solution to each well. Cover with the Microtiter plate sealers. Incubate for 1 hour at 37°C. Biotin-antibody working solution may appear cloudy. Warm to room temperature and mix gently until solution appears uniform.

5. Repeat the Aspirate/Wash four times.

6. Add 100µl of HRP-avidin working solution to each well. Cover the microtiter plate with a new adhesive strip. Incubate for 0.5 hours at 37°C.

7. Repeat the Aspirate/Wash four times.

8. Add 100ul TMB Substrate to each well. Mix gently, **protected from light** and incubates at 37°C for 10-20 min.

9. Add one drop (100 µl) of TMB Stop Solution to each well to stop the color reaction. If color change does not appear uniform, gently tap the plate to ensure thorough mixing.

10. Determine the optical density of each well within 5 minutes, using a microplate reader set to 450 nm.

CALCULATION

Average the duplicate readings for each standard, control, and samples and subtract the average zero standard optical density. Plot the optical density for the standards versus the concentration of the standards and draw the best curve. The data can be linearized by using log/log paper and regression analysis may be applied to the log transformation. To determine the sample concentration of each sample, first find the absorbance value on the y-axis and extend a horizontal line to the standard curve. At the point of intersection, extend a vertical line to the x-axis and read the corresponding sample concentration. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

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