LS Bio
an Absolute Biotech Company

Human IL8 / Interleukin 8 ELISA Development Kit (TMB)

Catalog No.: LS-F57506

DESCRIPTION

This kit includes the 4 principal components required to prepare approximately 200 sandwich ELISA plate wells for the quantitative measurement of Human IL8 / Interleukin 8 within the range of 1–150 pg/ml; the capture and detection antibodies, an antigen standard with which to create a standard curve and the streptavidin-HRP conjugate for detection. Below you will find our recommendations for all other materials needed, such as commercial reagents, recipes for solution preparation, instructions for coating the plates, and a recommended ELISA protocol.

Target Synonyms: IL8 / Interleukin 8, CXCL8, interleukin 8, 3-10C, AMCF-I, B-ENAP, C-X-C motif chemokine 8, GCP-1, IL-8, IL8, Interleukin-8, K60, LECT, GCP1, LUCT, NAF, NAP1, Neutrophil-activating factor, Interleukin 8, Protein 3-10C, MDNCF, TSG-1, Emoctakin, LYNAP, MONAP, NAP-1, SCYB8, T-cell chemotactic factor

RECONSTITUTION & STORAGE

Capture Antibody (lyophilized)**:** 5 µg of Rabbit anti-IL8 / Interleukin 8 capture antibody is supplied lyophilized with 0.5 mg D-mannitol. Centrifuge the vial prior to opening, then reconstitute with 50 µl sterile water for a concentration of 100 µg/ml.

Biotinylated Detection Antibody (lyophilized)**:** 6 µg of biotinylated Rabbit anti-IL8 / Interleukin 8 antibody is supplied lyophilized with 0.5 mg D-mannitol. Centrifuge the vial prior to opening, then reconstitute with 60 µl PBS containing 1% BSA for a concentration of 100 µg/ml.

Human IL8 / Interleukin 8 Standard (lyophilized)**:** 1 µg ofrecombinant IL8 / Interleukin 8 protein is supplied lyophilized with 2.2 mg BSA and 11 mg D-mannitol. To prepare a 1µg/ml Standard Stock Solution, centrifuge the vial prior to opening and reconstitute with 1000µl sterile water.

> ***Note****: Reconstituted antibodies and standards are stable for 2 weeks at 2-8°C or for up to 6 months at -20°C.*

Streptavidin-HRP Conjugate: The Streptavidin-HRP Conjugate is supplied as 4 µl liquid. Centrifuge the vial prior to opening, then dilute using 36 µl 1x PBS for a total of 40 µl at a concentration of 100 µg/ml. Prepare two 20µl aliquots for storage at 2-8°C for at least 6 months. Store in the dark and do not freeze. Use Streptavidin-HRP Conjugate with TMB only.

RECOMMENDED MATERIALS

- TMB Substrate Solution (KPL Cat. # 52-00-02)
- BSA (Sigma Cat # A-7030)
- Dulbecco's PBS [10x] (Gibco BRL Cat. # 14200-075)
- ELISA Plates, Strips (Nunc MaxiSorp Cat. #439454)
- Tween-20 (Sigma Cat. # P-7949)

RECOMMENDED SOLUTIONS

All solutions should be at ambient temperature prior to use.

- PBS: dilute 10x PBS to 1x PBS, pH 7.2 in sterile water
- Wash Buffer: 0.05% Tween-20 in PBS
- Block Buffer*: 1% BSA in 1x PBS
- Diluent*: 0.05% Tween-20, 0.1% BSA in 1x PBS

  * Sterile filter and store at 4°C for up to 1 week.

PLATE PREPARATION

1. Dilute the Capture Antibody to a concentration of 0.125 µg/ml with 1x PBS and immediately add 100 µl to each ELISA plate well. Seal the plate and incubate overnight at room temperature.
2. Aspirate each well and wash 4 times using 300 µl of Wash Buffer. Invert the plate on a paper towel to remove residual buffer.
3. Add 300 µl of Block Buffer to each well and incubate for at least 1 hour at room temperature.
4. Aspirate each well and wash 4 times using 300 µl of Wash Buffer.

ELISA PROTOCOL

5. **Standard/ Sample:** Using Diluent, prepare a serial dilution series of the IL8 / Interleukin 8 Standard Stock Solution from 150 pg/ml to 1 pg/ml. Use Diluent alone as a 0 pg/mlnegative control. Researchers must determine the optimal samples dilutions for their particular experiment. Using Diluent, prepare a dilution series of your unknown sample. Add 100 µl of standard or sample to each well in triplicate and incubate at room temperature for at least 2 hours.
6. **Detection:** Dilute the desired amount of Detection Antibody to a concentration of 0.25 µg/ml with Diluent. Aspirate each well and wash 4 times using 300 µl of Wash Buffer. Add 100 µl of Detection Antibody per well and incubate at room temperature for 2 hours.
7. **Streptavidin-HRP Conjugate:** Dilute the desired amount of Streptavidin-HRP Conjugate to a concentration of 100 µg/ml with Diluent. Aspirate each well and wash 4 times using 300 µl of Wash Buffer. Add 100 µl of Streptavidin-HRP Conjugate to each well and incubate 30 minutes at room temperature.
8. **TMB Liquid Substrate:** TMB Substrate Solution should be at ambient temperature prior to use. Aspirate each well and wash 4 times using 300 µl of Wash Buffer. Add 100 µl of TMB Substrate Solution to each well and incubate at room temperature for approximately 20 minutes. Monitor color development with an ELISA plate reader at 450nm with wavelength correction set at 620nm. Do not allow the O.D. readings for the zero standard to exceed 0.15. When optimal color development has been achieved add 100 µl of 1M HCL Stop Solution and take your final O.D. reading.
9. Use the O.D. values of the antigen standard serial dilution to generate a Standard Curve. This Standard Curve can then be used to calculate the concentration of the antigen in the unknown sample based on its O.D. value. See an example Standard Curve on the back side of this document. A Standard Curve should always be determined within each experiment.

SAMPLE ONLY

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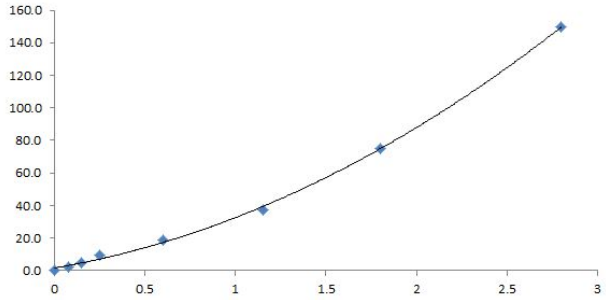
Tested Reactivity:
When tested at 50ng/ml the following antigens exhibited complete O.D. saturation. Human: IL-8 . When tested at 50ng/ml the following antigens did not exhibit significant cross reactivity. Human: BCA-1, BRAK, CXCL16, ENA-78, Fractalkine, GCP-2, GRO- alpha /MGSA, GRO- beta , GRO- gamma , I-TAC, IP-10, Lymphotactin, MCP-1, MIG, NAP-2, PF-4, SDF-1 alpha , SDF-1 beta.

Example Standard Curve: This LS-F57506 standard curve is an example only and may not be representative of this specific lot.



Important Note: During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. We recommend briefly centrifuging the vial to dislodge any liquid in the container's cap prior to opening.

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