

Product Datasheet



**Leinco
Technologies, Inc.**

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Human Eotaxin-3/CCL26 ELISA Development Kit

Prod. No.: E145
Lot No.: *Always Refer To Lot Specific Datasheet Provided With Kit for Accurate Instructions*
Pkg. Size: 15 plates, 45 plates

Description

Human Eotaxin-3 ELISA development kit contains the key components required for the quantitative measurement of natural and/or recombinant Eotaxin-3 in a sandwich ELISA format within the range of 32-3000 pg/ml. Using the ELISA protocol described below, this kit provides sufficient reagents to assay Eotaxin-3 in approximately 1500 ELISA plate wells.

Components Provided

Capture Antibody: 180 µg of antigen-affinity purified goat anti-Eotaxin-3. Centrifuge vial prior to opening. Reconstitute in 1.0 ml PBS for a concentration of 180 µg/ml. Following reconstitution the Capture antibodies may be stored at 2 – 8°C for up to 6 months. For long term storage it is recommended to aliquot into working volumes and store at -70°C in a manual defrost freezer. **Avoid repeated freeze and thaw cycles.**

Detection Antibody: 18 µg of biotinylated antigen-affinity purified goat anti-Eotaxin-3. Centrifuge vial prior to opening. Reconstitute in 1.0 ml sterile 1.0% BSA in PBS for a concentration of 18 µg/ml. Following reconstitution the Detection antibodies may be stored at 2 – 8°C for up to 6 months. For long term storage it is recommended to aliquot into working volumes and store at -70°C in a manual defrost freezer. **Avoid repeated freeze and thaw cycles.**

Human Eotaxin-3 Standard: 60 ng of recombinant Eotaxin-3. Centrifuge vial prior to opening. Reconstitute in 0.5 ml sterile 1.0% BSA in PBS for a concentration of 120 ng/ml. The Standard may be stored at 2 – 8°C for one month or aliquoted and stored at -70°C for up to three months in a manual defrost freezer. **Avoid repeated freeze and thaw cycles.**

UltraAvidin-HRP Conjugate: 40 µl vial. Upon receipt, UltraAvidin-HRP conjugate should be stored at 2 – 8°C, **DO NOT FREEZE**

TMB Liquid Substrate:

(TMB Substrate should be at ambient temperature prior to use)

Aspirate and wash plate 4 times. Add 100 µl of TMB HRPO Microwell Substrate Standard Kinetic One Component "Ready Use" (Leinco Prod. T118) to each well. Incubate at room temperature and monitor color 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.



Products are for research use only. Not for use in diagnostic or therapeutic procedures.

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Additional Required Materials

ELISA microplates (Thermo Fisher Cat. # 456529)
BSA (Sigma Cat. # A-7030)
Dulbecco's PBS [10x] (Gibco BRL Cat. # 14200-075)
Stop Solution 2 M Sulfuric Acid (Sigma Cat. # 339741)

Required 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*

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

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 300 µl of wash buffer per well.
3. After the last wash invert plate to remove residual buffer and blot on paper towel.
4. Add 300 µl block buffer to each well and incubate for at least 1 hour at room temperature.
5. Aspirate and wash plate 4 times.

ELISA Protocol

Standard/Sample: Dilute standard from 2000 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 1:5000 (*this dilution factor may require some optimization*) in diluent for a total volume of 15 ml. Add 100 µl 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" (Leinco Prod. T118) 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 (Sigma Cat. # 339741) to each well. *All eye, hand, face, and clothing protection should be used when working with sulfuric acid.*

NOTE: Reliable standard curves are obtained when either O.D. readings do not exceed 0.2 units for the zero standard concentrations or 1.6 units for the highest standard concentration. The plate should be monitored at 5 minute intervals for approximately 30 minutes. *O.D. readings may vary.*



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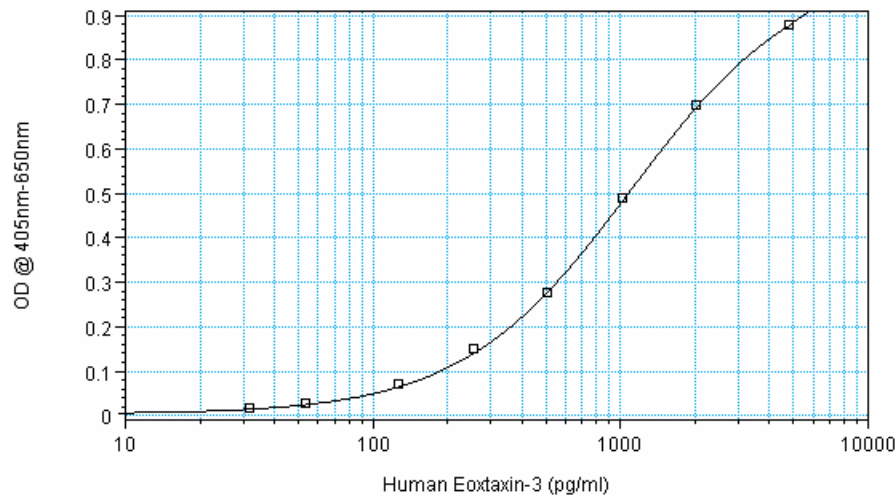
Cross Reactivity

When tested at 50 ng/ml the following antigen exhibited less than 5% cross reactivity:

Human Eotaxin, Eotaxin-2, MCP-1, MIP-1 α , - β , - Δ , MIP-3 α , - β , RANTES and mouse Eotaxin and Eotaxin-2.

Typical Standard Curve

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



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