

CCL11 Mouse Eotaxin ELISA Kit

Catalog No: CK422

Size: 1 x 96 wells

Introduction

The Cell Sciences® Mouse Eotaxin ELISA (Enzyme-Linked Immunosorbent Assay) kit is an *in vitro* enzyme-linked immunosorbent assay for the quantitative measurement of mouse Eotaxin in serum, plasma and cell culture supernatants. This assay employs an antibody specific for mouse Eotaxin coated on a 96-well plate. Standards and samples are pipetted into the wells and Eotaxin present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-mouse Eotaxin antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of Eotaxin bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm.

Reagents and materials supplied with the kit:

| Items | Quantity |
|---|--|
| A. Eotaxin Microplate coated with anti-mouse Eotaxin | 96 wells (12 strips x 8 wells) |
| B. Wash Buffer Concentrate (20x) | 25 ml |
| C. Recombinant mouse Eotaxin Standards | 2 vials |
| D. Assay Diluent A for Standard/Sample (serum/plasma) diluent* | 30 ml |
| E. Assay Diluent B (5x) for Standard/Sample (cell culture medium/urine) diluent | 15 ml |
| F. Detection Antibody-Biotinlyated anti-mouse Eotaxin | 2 vials (each vial is enough to assay half microplate) |
| G. Streptavidin-HRP Concentrate (4,000x) | 8 µl |
| H. TMB One-Step Substrate Reagent (TMB in buffered solution) | 12 ml |
| I. Stop Solution (2 M sulfuric acid) | 8 ml |

* Contains 0.09% sodium azide as preservative. Precaution: Sodium azide is a poisonous and hazardous substance which should be handled by trained staff only.



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Storage of Kit Reagents

Stable for up to 5 months from date of shipment at 2-4°C. Store reconstituted standard (recombinant protein) at -80 °C. Opened Microplate Wells and reagents are stable for 1 month at 2-4°C. Return unused wells to the pouch containing desiccant pack and reseal along the entire edge.

Materials/reagents required but not provided:

- Microplate reader capable of measuring absorbance at 450 nm
- Precision pipettes to deliver 2 µl to 1 ml volumes
- Adjustable 1-25 ml pipettes for reagent preparation
- 100 ml and 1 liter graduated cylinders
- Absorbent paper
- Distilled or deionized water
- Log-log graph paper or computer and software for ELISA data analysis
- Tubes to prepare standard or sample dilutions

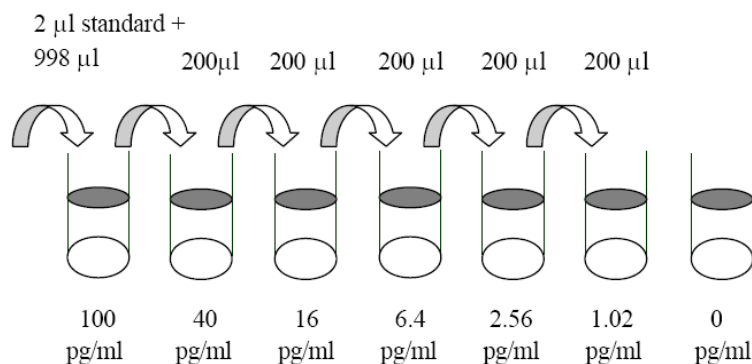
Preparation of Kit Reagents

Bring all reagents and samples to room temperature (18 - 25°C) before use.

Eotaxin Standard

- Briefly spin the vial of Item C (Recombinant Mouse Eotaxin Standard).
- Add 400 µl Assay Diluent A (for serum/plasma samples) or 1x Assay Diluent B (for cell culture medium) into Item C vial to prepare a 50 ng/ml standard.
- Dissolve the powder thoroughly by a gentle mix.
- Add 2 µl Eotaxin standard from the vial of Item C, into a tube with 998 µl Assay Diluent A or 1x Assay Diluent B to prepare a 100 pg/ml stock standard solution.
- Pipette 300 µl Assay Diluent A or 1x Assay Diluent B into each tube. Use the stock standard solution to produce a dilution series (shown below in Figure 1).
- Mix each tube thoroughly before the next transfer.
- Assay Diluent A or 1x Assay Diluent B serves as the zero standard (0 pg/ml).

Figure 1



Sample Dilution

If your samples need to be diluted, use Assay Diluent A (Item D) for dilution of serum/plasma samples, and Assay Diluent B (Item E) for dilution of culture supernatants and urine.

Assay Diluent B

Dilute 5-fold with deionized or distilled water.

Wash Buffer Concentrate

- If the Wash Concentrate (20x) (Item B) contains visible crystals, warm to room temperature and mix gently until dissolved.
- Dilute 20 ml of Wash Buffer Concentrate into deionized or distilled water to yield 400 ml of 1x Wash Buffer.

Detection Antibody

- Briefly spin Detection Antibody vial (Item F) before use.
- Add 100 µl of 1x Assay Diluent B into the vial to prepare a detection antibody concentrate.
- Pipette up and down to mix gently (the concentrate can be stored at 2-4°C for 5 days).
- The detection antibody concentrate should be diluted 65-fold with 1x Assay Diluent B and used in step 4 of the **ELISA Method**.

Streptavidin-HRP Concentrate

- Briefly spin Streptavidin-HRP Concentrate vial (Item G) and pipette up and down to mix gently before use.
- Streptavidin-HRP concentrate should be diluted 4,000-fold with 1x Assay Diluent B.
- *For example: Briefly spin the vial (Item G) and pipette up and down to mix gently. Add 3 µl of Streptavidin-HRP concentrate into a tube with 12 ml 1x Assay Diluent B to prepare a 4,000-fold diluted Streptavidin-HRP solution (don't store the diluted solution for next day use).*

ELISA Method

Be sure to read 'Preparation of Kit Reagents' before carrying out the assay.

1. Bring all reagents and samples to room temperature (18 - 25°C) before use. It is recommended that all standards and samples be run at least in duplicate.
2. Add 100 µl of each standard (see **Preparation of Kit Reagents: Eotaxin Standard**) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or overnight at 2-4°C.
3. Discard the solution and wash 4 times with 1x Wash Solution (200 µl each).
4. Add 100 µl of 1x prepared biotinylated antibody (see **Preparation of Kit Reagents: Detection Antibody**) to each well. Incubate for 1 hour at room temperature.
5. Discard the solution and wash 4 times with 1x Wash Solution (200 µl each).
6. Add 100 µl of prepared Streptavidin solution (see **Preparation of Kit Reagents: Streptavidin-HRP Concentrate**) to each well. Incubate for 45 minutes at room temperature.
7. Discard the solution and wash 5 times with 1x Wash Solution (200 µl each).



8. Add 100 μ l of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark.
9. Add 50 μ l of Stop Solution (Item I) to each well. Read at 450 nm immediately.

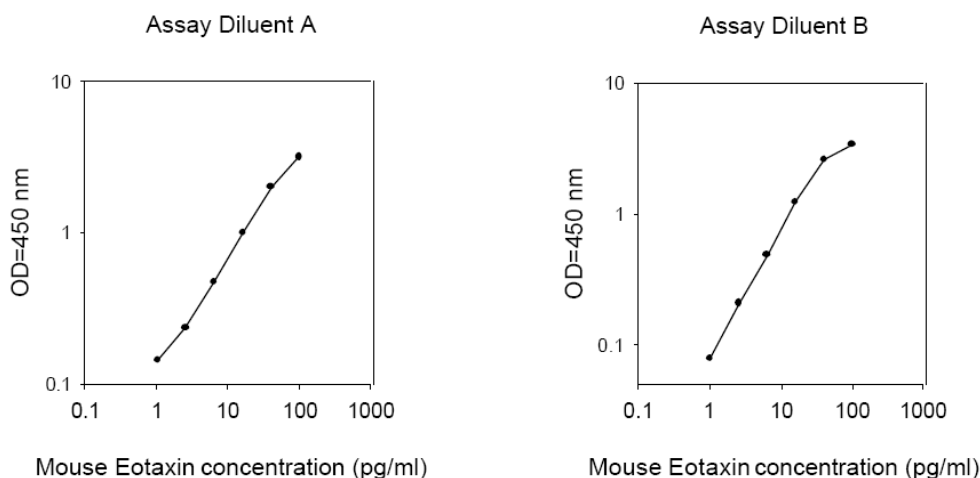
Calculation of Results

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

Figure 2

Typical Data

These standard curves are for demonstration only. A standard curve must be run with each assay.



Performances and Characteristics

Sensitivity

The minimum detectable dose of Eotaxin is typically less than 1 pg/ml.

Recovery

Recovery was determined by spiking various levels of recombinant mouse Eotaxin into mouse serum, plasma and cell culture media. Mean recoveries are as follows:

| Sample Type | Average % Recovery | Range (%) |
|--------------------|--------------------|-----------|
| Serum | 94.95 | 83-104 |
| Plasma | 92.54 | 84-103 |
| Cell culture media | 95.25 | 83-104 |



Linearity

| Sample Type | | Serum | Plasma | Cell Culture Media |
|-------------|---------------------------------|--------------|--------------|--------------------|
| 1:2 | Average % of Expected Range (%) | 93 84-104 | 92 83-104 | 94 84-104 |
| 1:4 | Average % of Expected Range (%) | 98 83-103 | 95 84-104 | 96 84-105 |
| 1:8 | Average % of Expected Range (%) | 97 85-103 | 95 83-103 | 95 83-103 |

Reproducibility

Intra-assay: CV<10%

Inter-assay: CV<12%

Specificity

Cross Reactivity: This ELISA kit shows no cross-reactivity with any of the cytokines tested (e.g., Mouse CD30, L CD30, T CD40, CRG2, CTACK, CXCL16, Eotaxin 2, Fas Ligand, Fractalkine, GCSF, GM-CFS, IGFBP3, IGFBP5, IGFBP6, IL1-alpha, IL1-beta, IL2, IL3, IL3 Rb, IL4, IL5, IL6, IL9, IL10, IL12 p40/p70, IL12 p70, IL13, IL17, KC, Leptin R, LEPTIN(OB), LIX, L-Selectin, Lymphotactin, MCP1, MCP5, M-CSF, MIG, MIP1a, MIP2, MIP3-beta, MIP3-alpha, PF4, P-Selectin, RANTES, SCF, SDF1-alpha, TARC, TCA3, TECK, TIMP1, TNF-alpha, TNF RI, TNF RII, TPO, VCAM1, VEGF).

Troubleshooting Guide

| Problem | Cause | Solution |
|------------------------|---|---|
| 1. Poor standard curve | 1. Inaccurate pipetting 2. Improper standard dilution | 1. Check pipettes 2. Ensure a brief spin of Item C and dissolve the powder thoroughly by a gentle mix. |
| 2. Low signal | 1. Too brief incubation times 2. Inadequate reagent volumes or improper dilution | 1. Ensure sufficient incubation time; ELISA Method Step 2 may change to overnight. 2. Check pipettes and ensure correct preparation. |
| 3. Large CV | 1. Inaccurate pipetting | 1. Check pipettes. |
| 4. High background | 1. Plate is insufficiently washed 2. Contaminated wash buffer | 1. Review the manual for proper wash. If using a plate washer, check that all ports are unobstructed. 2. Make fresh wash buffer. |
| 5. Low sensitivity | 1. Improper storage of the ELISA Kit 2. Stop solution | 1. Store your standard at < -20°C after reconstitution, others at 2-4°C. Keep substrate solution protected from light. 2. Stop solution should be added to each well before measure. |

NOT FOR HUMAN USE. FOR RESEARCH ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.



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