Signosis
Innovative Plate Assay Solutions

Human Eotaxin-3 ELISA

Catalog Number EA-0512 **(For Research Use Only)**

Introduction

Eotaxin-3, now also called chemokine (C-C motif) ligand 26 (CCL26) or macrophage inflammatory protein 4-alpha (MIP-4-alpha), is a cytokine belonging to the CC chemokine family. It attracts and activates eosinophils, basophils, and Th2 type T lymphocytes. It is expressed by several tissues including heart, lung and ovary, and in endothelial cells that have been stimulated with the cytokine interleukin 4.

Principle of the assay

Eotaxin-3 ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes goat anti-human Eotaxin-3 for immobilization on the microtiter wells and biotinated goatt anti-human Eotaxin-3 antibodies along with streptavidin conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with the two antibodies, resulting in the Eotaxin-3 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then stopped with the addition of Stop Solution changing the color to yellow. The concentration of Eotaxin-3 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

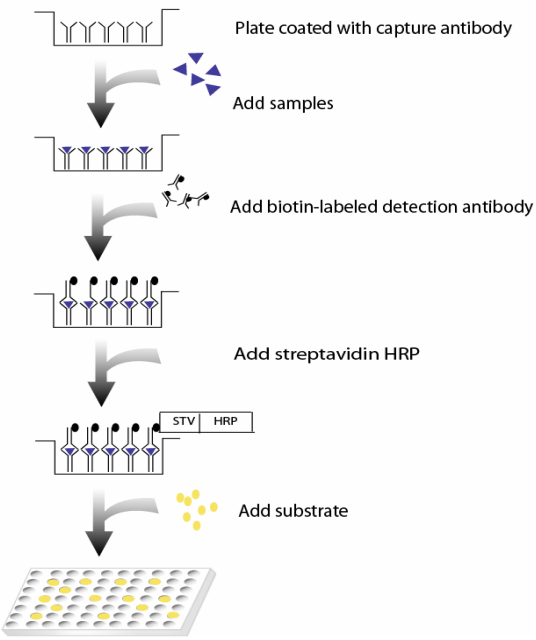


Diagram of ELISA

Materials provided with the kit

- 8x12 96-well microplate coated with goat anti-human Eotaxin-3 antibodies (4ºC).
- Biotin labeled goat anti-human Eotaxin-3 antibodies (-20ºC).
- Streptavidin-HRP conjugate (4ºC).
- Recombinant human Eotaxin-3 standard (400ng/ml) (-20ºC).
- 1X Diluent buffer (4ºC).
- 5X Assay wash buffer (4ºC)
- Substrate (4ºC).
- Stop Solution (4ºC).

Material required but not provided

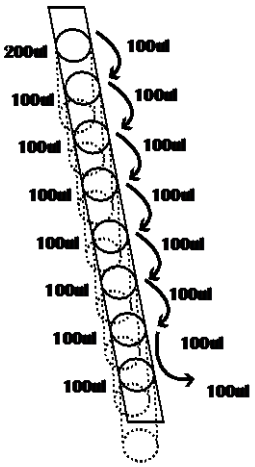
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Use serum-free conditioned media or original or 10-fold diluted sera. Sera can be diluted with 1 X Diluent buffer. When serum-containing conditioned media is required, be sure to use serum as a control.
- Dilute 100 times of human recombinant Eotaxin-3 (400ng/ml) with 1X Diluent buffer to 4000pg/ml and then 2-fold serial dilutions.
- Dilute 400 times of biotin labeled goat anti-human Eotaxin-3 antibodies with 1X Diluent buffer before use.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

Assay procedure

1. Calculate the number of samples to decide how many strips need to be used.
2. Add 100µl of Standard, control, or sample per well and incubate for 1 hour at room temperature with gentle shaking.



a. Add 200ul 1X Diluent buffer to the 1st well. Add 100ul 1X Diluent Buffer to the rest wells of strip.
b. Add appropriate amount of protein recombinant (follow instruction in "Reagent Preparation")
c. Mix dilutions in 1st well and transfer 100ul from the 1st well to the next dilution. (See picture) Incubate each well for 1 hr at room temperature with gentle shaking

3. Aspirate each well and wash by adding 200µl of 1X Assay wash buffer. Repeat the process three times for a total of three washes. Complete removal of liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.
4. Add 100 µl of diluted biotin-labeled goat anti-human Eotaxin-3 antibodies to each well and incubate for 1 hour at room temperature with gentle shaking.
5. Repeat the aspiration/wash as in step 3.
6. Add 100 µl of diluted streptavidin-HRP conjugate to each well and incubate for 45 min at room temperature with gentle shaking.
7. Repeat the aspiration/wash as in step 3.
8. Add 100µl of substrate to each well and incubate for 5-30 minutes.
9. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
10. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

Example of standard curve