

Human IL-13 ELISA

Catalog Number EA-0511

(For Research Use Only)

Introduction

Interleukin 13 (IL-13) is a cytokine that is an important mediator of allergic inflammation and disease. IL-13 is also important in allergic inflammation in many tissues. In addition, it plays functions in anti-inflammation. It is secreted by many cell types, but especially T helper type 2 (Th2) cells.

Principle of the assay

IL-13 ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes rabbit anti-human IL-13 for immobilization on the microtiter wells and biotinated rabbit anti-human IL-13 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 IL-13 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 IL-13 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

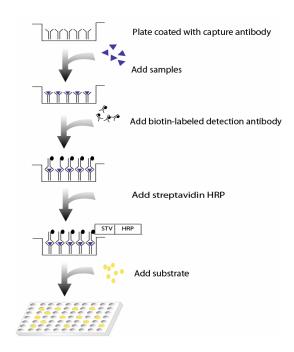


Diagram of ELISA

Materials provided with the kit

- 96 well microplate coated with rabbit antihuman IL-13 antibodies (4°C).
- Biotin labeled rabbit anti-human IL-13 antibodies (-20°C).
- Streptavidin-HRP conjugate (4°C).
- Recombinant human IL-13 standard (1000ng/ml) (-20°C).
- 1X Diluent buffer (4°C).
- 5X Assay wash buffer (RT)
- Substrate (4°C).
- Stop Solution (4°C).

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
- Dilute 500 times of human recombinant IL-13 (1000ng/ml) with 1X Diluent buffer to 2000pg/ml and then 2-fold serial dilutions.
- Dilute 400 times of biotin labeled rabbit anti-human IL-13 antibodies with 1X Diluent buffer before use.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

Assay procedure

- 1. Cut the sealing film over the plate and remove it from the desired number of wells. Make sure the rest of wells are well sealed.
- 2. Add $100 \square$ µl of Standard, control, or sample per well and incubate for 1 hour at room temperature with gentle shaking.
- 3. Aspirate each well and wash by adding $200\mu l$ 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 μ l of diluted biotin-labeled rabbit anti-human IL-13 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 $100\mu l$ of substrate to each well and incubate for 5-30 minutes.
- 9. Add $50\mu l$ 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

