

Mouse IL-12 ELISA

Catalog Number EA-2514

(For Research Use Only)

Introduction

Interleukin 12 (IL-12) is an interleukin that plays important roles in the activities of natural killer cells and T lymphocytes, the differentiation of naive T cells into Th1 cells, the growth and function of T cells, the production of interferon-gamma (IFN- γ) and tumor necrosis factor-alpha (TNF- α) from T and natural killer (NK) cells, and IL-4 mediated suppression of IFN- γ . It also plays a role in anti-angiogenic activity by increasing production of IFN- γ , which in turn increases the production of IP-10. IL-12 is naturally produced by dendritic cells, macrophages and mouse B-lymphoblastoid cells in response to antigenic stimulation.

Principle of the assay

IL-12 ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes goat anti-mouse IL-12 for immobilization on the microtiter wells and biotinated goat anti-mouse IL-12 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-12 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unboundlabeled 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-12 is directly proportional to the color intensity of Absorbance the test sample. is measured spectrophotometrically at 450 nm.

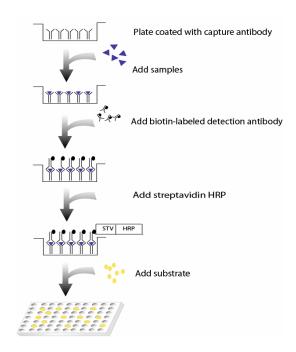


Diagram of ELISA

Materials provided with the kit

- 96 well microplate coated with goat antimouse IL-12 antibodies (4°C)
- Biotin labeled goat anti-mouse IL-12 antibodies (-20°C)
- Streptavidin-HRP conjugate (4°C)
- Recombinant mouse IL-12 standard (-20°C)
- 1X Diluent buffer (4°C)
- 5X Assay wash buffer (4°C)
- 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 400 times of biotin labeled goat anti-mouse IL-12 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 100µl 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 µl of diluted biotin-labeled goat anti-mouse IL-12 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.