Signosis
Innovative Plate Assay Solutions

Human IL-10 ELISA

Catalog Number EA-0513 **(For Research Use Only)**

Introduction

Interleukin-10(IL-10) is a cytokine primarily produced by monocytes and lymphocytes with important immunoregulatory functions. IL-10 can inhibit the synthesis of many pro-inflammatory cytokines including TNF-α, IL-6, and IL-1, and increase the transcription of anti-apoptotic genes. Mutations in IL-10 are associated with increased susceptibility to infection and rheumatoid arthritis. Understanding the conditions that alter the expression of this vital cellular messenger is important for unraveling the mechanisms of these and other diseases and for developing therapeutics.

Principle of the assay

IL-10 ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes rabbit anti-human IL-10 for immobilization on the microtiter wells, and biotinated rabbit anti-human IL-10 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-10 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 terminated with the addition of Stop Solution changing the color to yellow. The concentration of IL-10 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

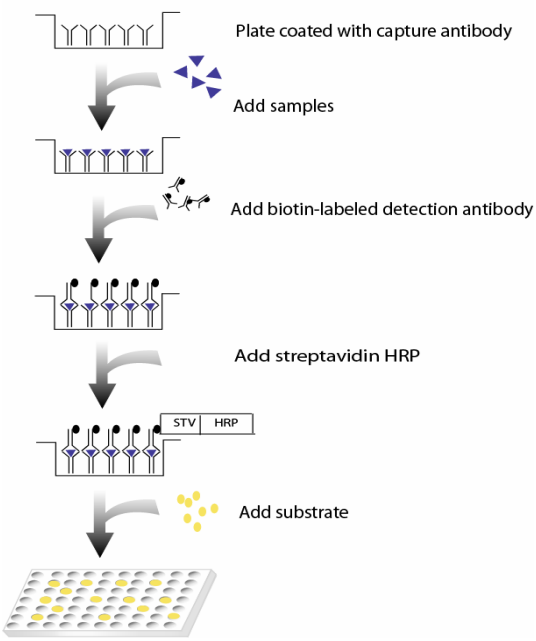


Diagram of ELISA

Materials provided with the kit

- 96 well microplate coated with rabbit anti-human IL-10 antibodies (4°C)
- Biotin labeled rabbit anti-human IL-10 antibodies (-20°C)
- Streptavidin-HRP conjugate (4°C)
- Recombinant human IL-10 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

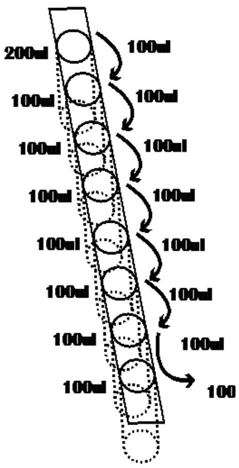
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute biotin labeled rabbit anti-human IL-10 antibodies 1:400 with 1X Diluent buffer before use.
- Dilute streptavidin-HRP 1:200 with 1X Diluent buffer before use.

Assay procedure

1. Cut the sealing film over the plate and remove it from the desired number of well strips. Make sure the rest of wells are well sealed.
2. Prepare standard according to diagram.



1. Add 200 µl 1X Diluent buffer to the 1st well. Add 100 µl 1x Diluent Buffer to the rest of the wells in the strip.
2. Add 8 µl of standard to the first well.
3. Mix dilution in 1st well and transfer 100 µl from the first well to the 2nd well.
4. Repeat mix and transfer 100 µl into each additional well as pictured.

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