



## Mouse 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- $\alpha$ , 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-mouse IL-10 for immobilization on the microtiter wells, and biotinylated rabbit anti-mouse 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.

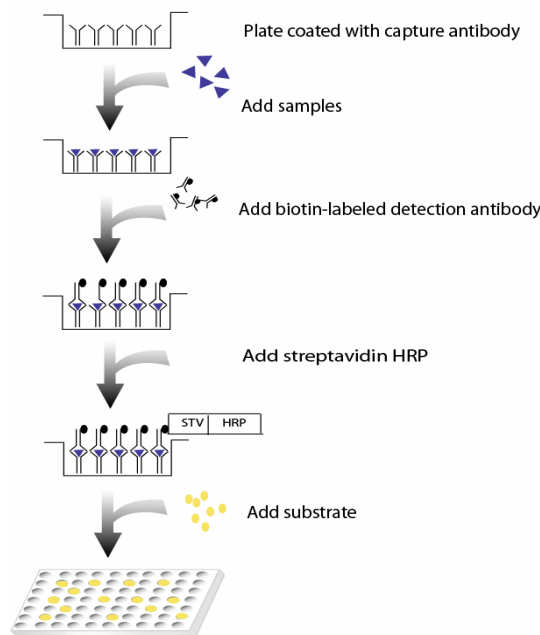


Diagram of ELISA

### Materials provided with the kit

- 8x12 96-well microplate coated with rabbit anti-mouse IL-10 antibodies (4°C)
- Biotin labeled rabbit anti-mouse IL-10 antibodies (-20°C)
- Streptavidin-HRP conjugate (4°C)
- Recombinant mouse 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 ddH<sub>2</sub>O
- Dilute 50 times of mouse recombinant IL-10 (200ng/ml) with 1X Diluent buffer to 4000pg/ml and then 2-fold serial dilutions. Add 4ul Mouse Recombinant IL-10 in 200ul 1X Diluent Buffer (See Step 2 below for detailed instruction)
- 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 biotin labeled rabbit anti-mouse IL-10 antibodies 1:400 with 1X Diluent buffer before use.
- Dilute streptavidin-HRP 1:200 with 1X Diluent buffer before use.

7. Add 100  $\mu$ l 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.

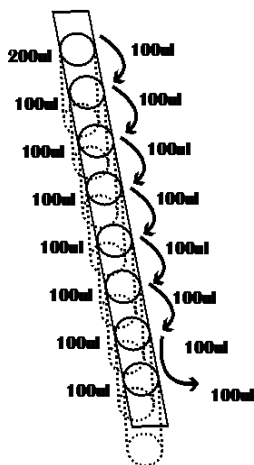
9. Add 100  $\mu$ l of substrate to each well and incubate for 10-30 minutes.

10. Add 50  $\mu$ l 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.

## Assay procedure

1. Calculate the number of strips need to be used
2. Prepare standard acc



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

3. Add 100  $\mu$ l of sample per well and incubate for 1 hour at room temperature with gentle shaking.
4. 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. 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  $\mu$ l of diluted biotin-labeled rabbit anti-mouse 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.