



## Human IP-10 ELISA

Catalog Number EA-0505

(For Research Use Only)

### Introduction

IFN-gamma-inducible protein (IP-10) is a member of the chemokine family of cytokines and is induced in a variety of cells in response to interferon gamma and lipopolysaccharide. It is secreted by a number of cells including monocytes, endothelial cells and fibroblasts. IP-10 plays several roles, such as chemoattraction for monocytes/macrophages, T cells, NK cells, and dendritic cells, promotion of T cell adhesion to endothelial cells, antitumor activity, and inhibition of bone marrow colony formation. Several cell types in response to IFN- $\gamma$ . IP-10 acts as potent inhibitors of angiogenesis in vivo.

### Principle of the assay

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

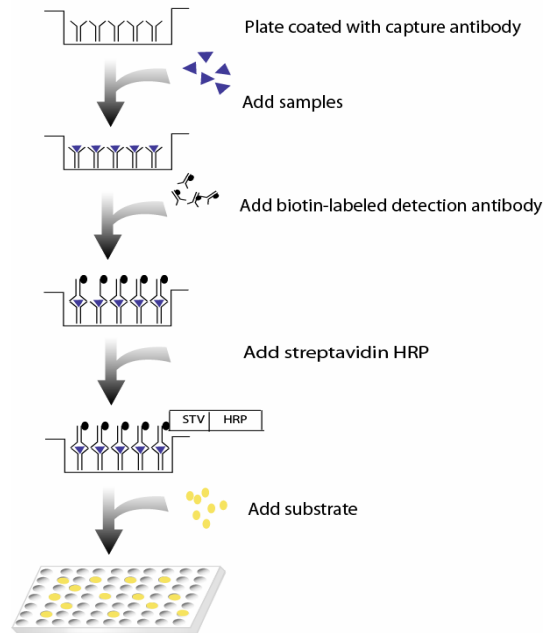


Diagram of ELISA

### Materials provided with the kit

- 96 well microplate coated with rabbit anti-human IP-10 antibodies (4°C).
- Biotin labeled rabbit anti-human IP-10 antibodies (-20°C).
- Streptavidin-HRP conjugate (4°C).
- Recombinant human IP-10 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 ddH<sub>2</sub>O
- Dilute 500 times of human recombinant IP-10 (1000ng/ml) with 1X Diluent buffer to 2000pg/ml and then 2-fold serial dilutions.
- Dilute 400 times of biotin labeled rabbit anti-human IP-10 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  $\mu$ l 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  $\mu$ l of diluted biotin-labeled rabbit anti-human IP-10 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  $\mu$ l 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

