Signosis
Innovative Plate Assay Solutions

Rat IP-10 ELISA

Catalog Number EA-3012 **(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-γ. 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 anti-rat IP-10 for immobilization on the microtiter wells and biotinylated anti-rat 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.

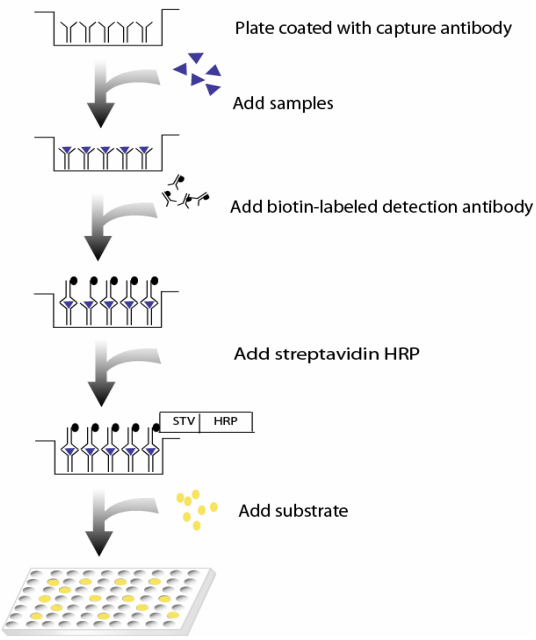


Diagram of ELISA

Materials provided with the kit

- 8x12 96-well microplate coated with anti-rat IP-10 antibodies (4ºC).
- Biotin labeled anti-rat IP-10 antibodies (-20ºC).
- Streptavidin-HRP conjugate (4ºC).
- Recombinant rat IP-10 standard (-20ºC).
- 1X Diluent buffer (4ºC).
- 5X Assay wash buffer (4ºC)
- Substrate (4ºC).
- Stop Solution (4ºC).

Material required but not provided

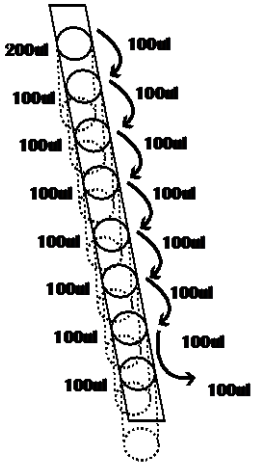
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Use serum-free conditioned media or original or 10-fold diluted sera. Sera can be diluted with 1x Diluent buffer. When serum-containing conditioned media is required, be sure to use serum as a control.
- Dilute 50 times of Rat recombinant IP-10 (400ng/ml) with 1x Diluent buffer to 8ng/ml by adding 4µl Rat IP-10 recombinant protein in first well with 200µl Diluent buffer then 2-fold serial dilutions (see step 2 in Assay procedure below for the detailed instruction).
- Dilute 400 times of biotin labeled anti-rat IP-10 antibodies with 1x Diluent buffer before use.
- Dilute 200 times of streptavidin-HRP with 1x Diluent buffer before use.
- Avoid contact of Substrate and Stop Solution with any metal surfaces.
- Important Note: Before beginning your experiment, quickly take 50µL of the Substrate and check to make sure it's clear. **If its color has changed to dark blue, do not begin the experiment and contact us immediately.**

Assay procedure

1. Calculate the number of samples to decide how many strips need to be used
2. See instruction and diagram below for standard preparation.



a. Add 200ul 1X Diluent buffer to the 1st well. Add 100ul 1X Diluent Buffer to the rest wells of strip.
b. Add appropriate amount of protein recombinant (follow instruction in "Reagent Preparation")
c. Mix dilutions in 1st well and transfer 100ul from the 1st well to the next dilution. (See picture) Incubate each well for 1 hr at room temperature with gentle shaking

3. Add 100ul of sample per well and incubate for 1 hour at room temperature with gentle shaking.
4. Aspirate each well and wash by adding 200µl 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.
5. Add 100µl of diluted biotin-labeled anti-rat IP-10 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. Before adding substrate, check to make sure it is clear. **If the substrate is already blue, please leave the wash buffer in the wells, seal and store the plate at 4°C, and contact us immediately.**
10. Add 100µl of substrate to each well and incubate for 10-30 minutes.
11. Add 50µl of Stop solution to each well. The color in the wells should change from blue to yellow.
12. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.