Signosis
Innovative Plate Assay Solutions

Rat IFNγ ELISA

Catalog Number EA-3003 **(For Research Use Only)**

Introduction

Interferons (IFNs) are potent extracellular protein mediators of host defense and homoeostasis. They are cytokines produced by the cells of the immune system in response to challenges by foreign agents such as viruses, parasites and tumor cells. It is produced by a wide variety of cells in response to the presence of double-stranded RNA, a key indicator of viral infection. IFNs are divided into two major subgroups. Type I IFNs all bind to a type I IFN receptor, such as IFN-α and IFN-β. IFN-γ is the sole type II IFN, which binds to a distinct type II receptor. Almost all cell types produce type I IFNs, while the type II IFN-γ is produced in T cells and natural killer (NK) cells upon immunological stimulation. IFN-γ coordinates a diverse array of cellular programs through transcriptional regulation of immunologically relevant genes. Cellular effects of IFN-γ includes up-regulation of pathogen recognition, antigen processing and presentation, the antiviral state, inhibition of cellular proliferation and effects on apoptosis, activation of microbicidal effector functions, immunomodulation, and leukocyte trafficking.

Principle of the assay

IFNγ ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes rabbit anti-rat IFNγ antibodies for immobilization on the microtiter wells and rabbit anti-rat IFNγ 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 IFNγ 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 IFNγ is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

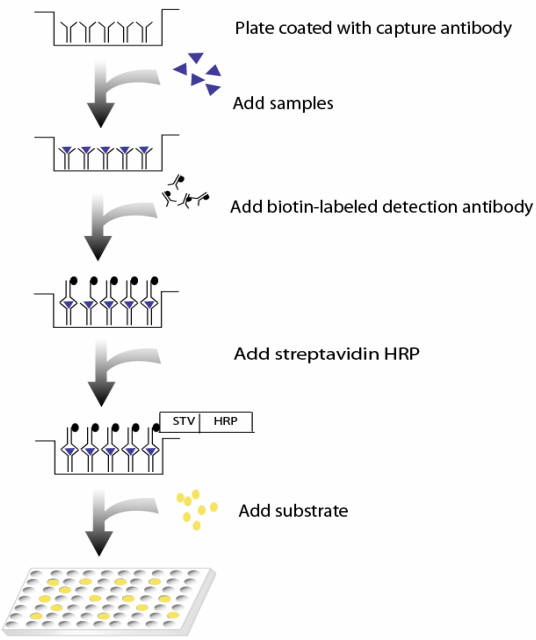


Diagram of ELISA

Materials provided with the kit

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

Material required but not provided

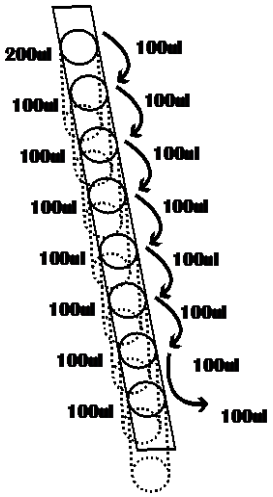
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

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 1 X Diluent buffer. When serum-containing conditioned media is required, be sure to use serum as a control.
- Dilute100 times of Rat recombinant IFNγ (400ng/ml) with 1X Diluent buffer to 4000pg/ml and then 2-fold serial dilutions.
  Add 4ul rat recombinant IFNγ in 200ul 1X Diluent Buffer (See Step 2 below for detailed instruction)
- Dilute 400 times of biotin labeled rabbit anti-rat IFNγ antibodies with 1X Diluent buffer before use.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

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 IFNr 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 100µl of substrate to each well and incubate for 10-30 minutes.
10. Add 50µl 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.