Signosis
Innovative Plate Assay Solutions

Rat FGFb ELISA

Catalog Number EA-3009 **(For Research Use Only)**

Introduction

Fibroblast growth factor (FGF)-2, also known as basic FGF (FGFb), is a powerful stimulator of angiogenesis in vivo (1), in addition to being a pleiotropic regulator of the proliferation, migration, differentiation, and survival of many cell types in vitro, including endothelial cells, smooth muscle cells, and pericytes. It is capable of inducing an angiogenic response both in vivo and in vitro (2,3). Both basic and acidic FGFs, a heparin-binding family of structurally related cytokines, are widely expressed during embryonic development and normal wound healing, as well as in such angioproliferative diseases as cancer and diabetes.

Principle of the assay

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

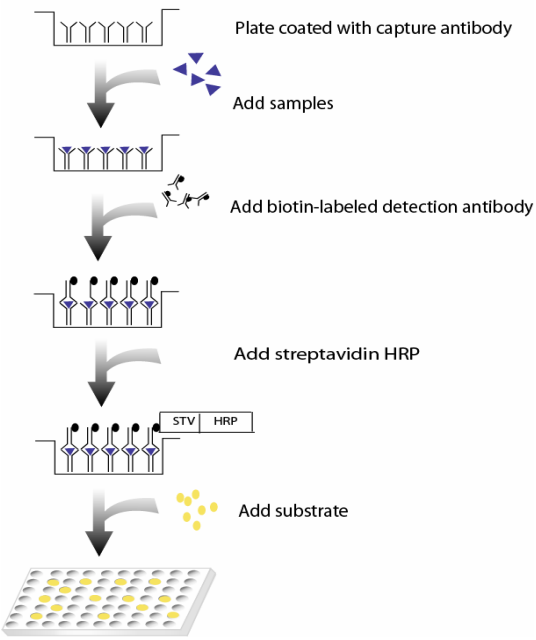


Diagram of ELISA

Materials provided with the kit

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

Material required but not provided

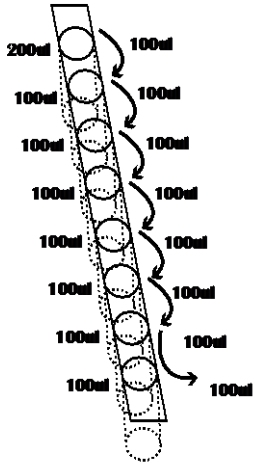
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

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 1 X Diluent buffer. When serum-containing conditioned media is required, be sure to use serum as a control.
- Dilute 50 times of Rat recombinant FGFb (200ng/ml) with 1X Diluent buffer to 4000pg/ml and then 2-fold serial dilutions.
  Add 4ul Rat recombinant FGFb in 200ul 1X Diluent Buffer (See Step 2 below for detailed instruction)
- Dilute 400 times of biotin labeled anti-rat FGFb antibody 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 FGFb 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.

Example of standard curve