Signosis
Innovative Plate Assay Solutions

Human TGF-β3 ELISA

Catalog Number EA-0209 **(For Research Use Only)**

Introduction

Transforming growth factor – beta isoforms, TGF-β1, TGF-β2, and TGF-β3play an important role in tissue homeostasis as wells as embryogenesis. Although these three isoforms share similar amino acid sequence and have similar bioactivities in *vitro*, they behave distinctly in *vivo*. Knock-out studies have shown that TGF-β1is responsible for angiogenesis while TGF-β2is needed for organ developments and TGF-β3is necessary for normal palate development. In tumorigenesis, three isoforms can be observed to be regulated differently, for example, TGF-β1is up-regulated at 3-8 fold while TGF-β3is down-regulated at 2-3 fold in mammary gland tumor cells. Signosis's TGF-beta ELISA kits can simultaneously analyze TGF-β1 or TGF-β3. Each well is coated with a specific capture antibody to detect corresponding cytokine in the sample. The expression levels of these cytokines can be quantitatively compared between samples.

Principle of the assay

TGF-β1/3 ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes a mouse anti-human TGF-β1/3 antibody for immobilization on the microtiter wells and chicken anti-human TGF-β1/3 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 TGF-β1/3 molecules being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. An 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 from blue to yellow. The concentration of TGF-β1/3 is directly proportional to the color intensity of the test sample. Absorbance is measured spectrophotometrically at 450 nm.

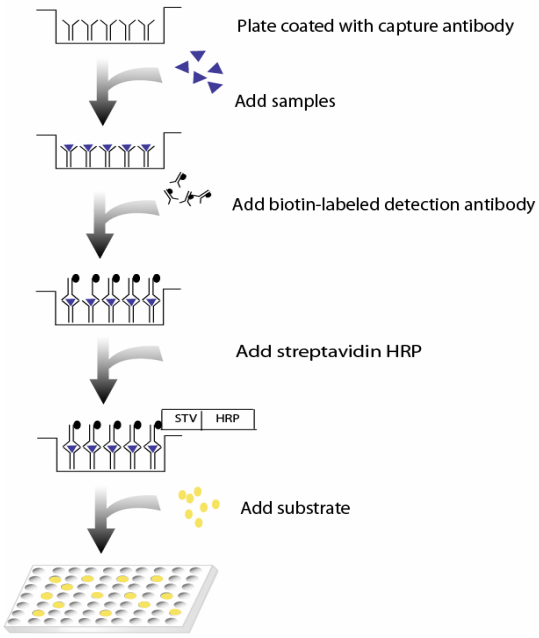


Diagram of ELISA

Materials provided with the kit

Component	Qty	Store at
8X12 96-well 12 strip plate coated with anti-human TGFβ3 antibody	1	4°C
Biotin labeled anti-human TGFβ3 antibody	25µL	-20°C
Recombinant Human TGFβ3 standard (400ng/ml)	10µL	-20°C
Streptavidin-HRP conjugate	50µL	4°C
1X Diluent buffer	40mL	4°C
5X Assay wash buffer	40mL	4°C
Substrate	10mL	4°C
Stop solution	5mL	4°C

Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.
- 1N HCl Solution
- 1.2N NaOH/0.5M HEPES Solution

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 100 times of Human recombinant TGF-β3 (400ng/ml) to 4000pg/ml by adding 2µl Human recombinant TGF-β3 in 200µl 1x Diluent Buffer and then 2-fold serial dilutions (See Step 2 below for detailed instruction
- Dilute 400 times of biotin labeled anti-human TGF-β3 antibody with 1X Diluent buffer before use.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

TGF-β 1/3 Sample Activation

TGF-β1/3 in biological samples exist in one of two forms: latent or free TGF-β1/3. Signosis TGF-β1/3 ELISA kit allows detection of free, or immunoactive TGF-β1/3. To activate latent TGF-β1/3, follow activation procedure below by **using polypropylene test tubes**.
Note: Protein Standard within the kit *DOES NOT* require activation as it is already in active form.

For Cell Culture Assay:

1. Add 20ul of 1N HCl to 100ul cell culture supernatant, mix thoroughly.
2. Incubate for 10 minutes at room temperature.
3. Add 20ul of 1.2N NaOH/0.5M HEPES, mix thoroughly.
4. Proceed to assay procedure immediately.
5. The concentration reading from standard curve must be multiplied by a dilution factor of 1.4.

For Serum and Plasma Assay:

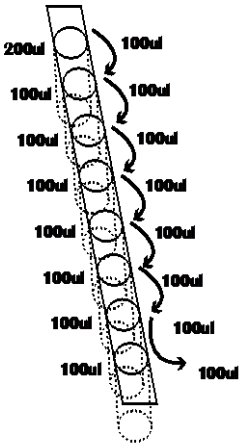
1. Add 20ul of 1N HCl to 40ul serum/plasma, mix thoroughly
2. Incubate for 10 minutes at room temperature.
3. Add 20ul of 1.2N NaOH/0.5M HEPES, mix thoroughly.
4. 20-fold dilution of activated sample before assay is recommended. For 20-fold dilution, mix 10ul activated sample and 190ul of Diluent buffer.
5. The concentration reading from standard curve must be multiplied by a dilution factor of 40.

Recommendation:

Signosis TGF-β1/3 ELISA kit allows detection of free TGF-β1/3. To measure latent TGF-β1/3, performing the same assay twice, one with activation and one without, is recommended. The difference in concentration from these two assays indicates concentration of TGF-β1/3.

Assay procedure

1. Calculate the number of samples to decide how many strips need to be used.
2. Add 100µl of Standard, control, or sample per well and incubate for 1 hour at room temperature with gentle shaking.



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. Aspirate each well and wash by adding 200µl 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.
4. Add 100µl of diluted biotin-labeled anti-human TGF-β3 antibody 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 µl 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 100µl substrate to each well and incubate for 5-30 minutes.
9. Add 50µl 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.