



# Mouse TGF-β1 ELISA

Catalog Number EA-2521

(For Research Use Only)

## Introduction

Transforming growth factor – beta isoforms, TGF-β1, TGF-β2, and TGF-β3 play an important role in tissue homeostasis as well 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-β1 is responsible for angiogenesis while TGF-β2 is needed for organ developments and TGF-β3 is necessary for normal palate development. In tumorigenesis, three isoforms can be observed to be regulated differently, for example, TGF-β1 is up-regulated at 3-8 fold while TGF-β3 is 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-Mouse TGF-β1/3 antibody for immobilization on the microtiter wells and chicken anti-Mouse 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.

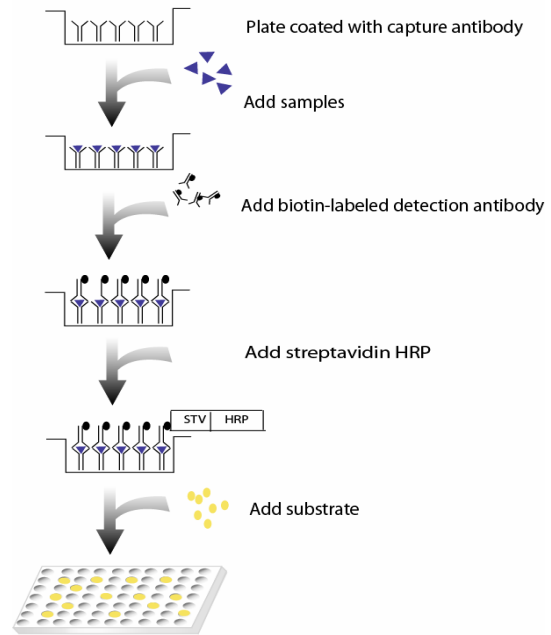


Diagram of ELISA

## Materials provided with the kit

Component	Qty	Store at
8X12 96-well 12 strip plate coated with anti-Mouse TGFβ1 antibody	1	4°C
Biotin labeled anti-Mouse TGFβ1 antibody	25μL	-20°C
Recombinant Mouse TGFβ1 standard (200ng/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 ddH<sub>2</sub>O
- 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 Mouse recombinant TGF- $\beta$ 1 (200 ng/ml) to 4000 pg/ml by adding 4 $\mu$ l Mouse recombinant TGF- $\beta$ 1 in 200 $\mu$ l 1x Diluent Buffer and then 2-fold serial dilutions (See Step 2 below for detailed instruction)
- Dilute 400 times of biotin labeled anti-Mouse TGF- $\beta$ 1 antibody with 1X Diluent buffer before use.
- Dilute 200 times of streptavidin-HRP with 1X Diluent buffer before use.

## TGF- $\beta$ 1/3 Sample Activation

TGF- $\beta$ 1/3 in biological samples exist in one of two forms: latent or free TGF- $\beta$ 1/3. Signosis TGF- $\beta$ 1/3 ELISA kit allows detection of free, or immunoactive TGF- $\beta$ 1/3. To activate latent TGF- $\beta$ 1/3, 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 20 $\mu$ l of 1N HCl to 100 $\mu$ l cell culture supernatant, mix thoroughly.
2. Incubate for 10 minutes at room temperature.
3. Add 20 $\mu$ l 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:

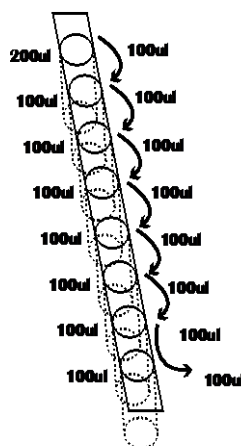
1. Add 20 $\mu$ l of 1N HCl to 40 $\mu$ l serum/plasma, mix thoroughly
2. Incubate for 10 minutes at room temperature.
3. Add 20 $\mu$ l of 1.2N NaOH/0.5M HEPES, mix thoroughly.
4. 20-fold dilution of activated sample before assay is recommended. For 20-fold dilution, mix 10 $\mu$ l activated sample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

### Recommendation:

Signosis TGF- $\beta$ 1/3 ELISA kit allows detection of free TGF- $\beta$ 1/3. To measure latent TGF- $\beta$ 1/3, 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- $\beta$ 1/3.

## Assay procedure

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



- a. Add 200 $\mu$ l 1X Diluent buffer to the 1<sup>st</sup> well. Add 100 $\mu$ l 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 1<sup>st</sup> well and transfer 100 $\mu$ l from the 1<sup>st</sup> 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 200 $\mu$ l 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 100 $\mu$ l of diluted biotin-labeled anti-Mouse TGF- $\beta$ 1 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  $\mu$ 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 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.