Signosis
Innovative Plate Assay Solutions

QuantiTF Smad1 ELISA Kit (Colorimetric)

Catalog Number QE-0001 **(For Research Use Only)**

Introduction

Smad transcription factors lie at the center of the transforming growth factor-beta (TGF-β) pathway, which is one of the most important cytokines signaling pathways. Members of the transforming growth factor-beta (TGF-β) superfamily bind to serine/threonine kinase receptors and specifically activate intracellular Smad proteins. Smads 2 and 3 are activated by activin/nodal and TGF-β, whereas Smads 1, 5 and 8 are activated by TGF-β-like BMP (Bone morphogenetic proteins). Smads family can be subsequently classified based on their activation by TGF-β or BMP cytokine family. These activated Smads form the complexes with co-Smads proteins, translocate from cytoplasm into nucleus and bind to the distinctive consensus binding sequences on the target promoter region to regulate the transcription of genes. Signosis has developed the QuantiTF Smad1 ELISA kit to facilitate the study of TGF-β/Smad pathway and specific analysis of the activities of Smad1 quantitatively in a high throughput way. The kit can be used for human, mouse and rat samples.

Principle of the assay

QuantiTF Smad1 ELISA kit is highly sensitive and specific assay with a simple and optimized procedure. The 96-well (12 Strips) clear plate is pre-immobilized with the Smad consensus sequencing oligo. The activated Smad in nuclear extract or the whole cell lysate is added in the well and binds to the oligo. The activated Smad1 is detected with a specific antibody against Smad1 subunit and a HRP conjugated secondary antibody. The assay utilizes colorimetric detection method, which can be easily measured by spectrophotometry. In the kit, we also provide Smad1 recombinant protein standard so that the activity of Smad1 in cells can be quantitatively studied.

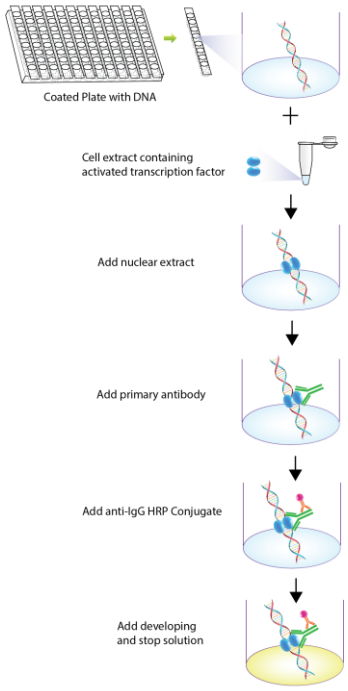


Diagram of TF ELISA

Materials provided with the kit

- 96-well (8x12) microplate coated with Smad3 consensus oligo (4ºC)
- Primary antibody against Smad3 (4ºC)
- HRP conjugate secondary antibody (4ºC)
- 2X TF binding buffer (-20ºC)
- 1X Nuclear extract dilution buffer (-20ºC)
- Smad1 recombinant protein 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
- Dilute 200 times of antibody against Smad1 with 1X Diluent buffer before use.
- Dilute 1000 times of HRP conjugate secondary antibody with 1X Diluent buffer before use.

Assay procedure

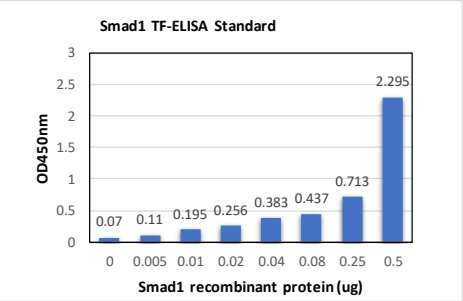
1. Calculate the number of samples to decide how many strips need to be used.
2. Make TF binding mix as following:

   25ul 2X TF binding buffer
   X Nuclear extract (2-10ug)
   X Nuclear extract dilution buffer
   Total 50ul

   For standard protein serial dilution preparation, please see the instruction in the end of this manual.
3. Add the reaction mix on a well and incubate for 1-2 hours without shaking at room temperature.
4. Discard the solution and wash by adding 200µl of 1X Assay wash buffer. Repeat the process 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 antibody against Smad to each well and incubate for 1.5 hour at room temperature with gentle shaking.
6. Repeat the aspiration/wash as in step 4 for a total of three washes.
7. Add 100 µl of diluted HRP conjugate secondary antibody to each well and incubate for 45 min at room temperature with gentle shaking.
8. Repeat the aspiration/wash as in step 4 for a total of three washes.
9. Add 100µl of substrate to each well and incubate for 30 minutes. The solution will gradually change to blue.
10. Add 50µl of stop solution to each well. The solution 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 Smad3 standard graph

Assay procedure



❖ **Recombinant protein standard for TF-ELISA:**

1. Add appropriate amount of recombinant protein standard to the first well as following:

   50ul 2X TF binding buffer
   Xul Recombinant protein (50ng)
   Xul Nuclear extract dilution buffer
   Total 100ul

2. Add 50ul of Nuclear extract dilution buffer to 2nd -7th wells in the same strips. Leave the 8th as blank.
3. Transfer 50ul solution from the 1st well to the next dilution well.
4. Repeat the transfer until the 7th well (Do not transfer to the 8th). Trash 50ul of the solution from the 7th well.
5. Use the 8th well as blank, adding H_2O instead of protein in the above reaction. Mix and only add 50ul in the 8th well.