Signosis
Innovative Plate Assay Solutions

Smad2/3 ELISA Kit (Colorimetric)

Catalog Number TE-0011 **(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 cytokine 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, 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 Smad2/3 ELISA kit to facilitate the study of TGF-β/Smad pathway.

Principle of the assay

Smad2/3 ELISA kit is high 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 Smad2/3 is detected with a specific antibody against Smad2/3 subunit and a HRP conjugated secondary antibody. The assay utilizes colorimetric detection method, which can be easily measured by spectrophotometry.

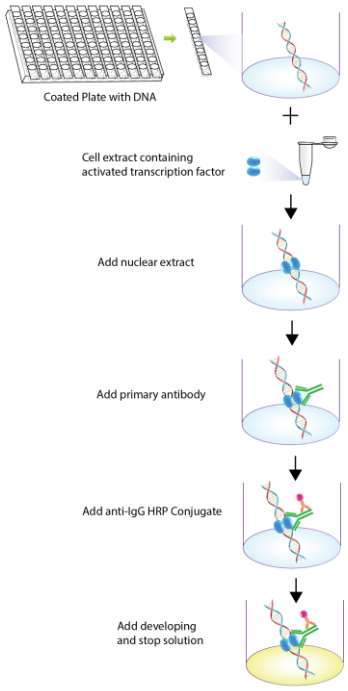

Diagram of TF ELISA

Materials provided with the kit

- 8x12 96-well microplate coated with Smad2/3 consensus oligo (4ºC).
- Antibody against Smad2/3 (4ºC).
- HRP conjugate secondary antibody (4ºC)
- 2X TF binding buffer (-20ºC).
- Nuclear extract dilution buffer (-20ºC).
- Smad2/3 positive control (-80ºC)
- 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 100 times of antibody against Smad2/3 with 1X Diluent buffer before use.
- Dilute 500 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
   30ul 2X TF binding buffer
   X Nuclear extract (2-10ug)
   X Nuclear extract dilution buffer
   Total 60ul
   For the positive control, add 30ul positive control nuclear extract provided.
3. Add the mix on a well and incubate at room temperature for 1-2 hours (or overnight at 4°C) without shaking.
4. Invert and discard the contents 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.
5. Add 60µl of diluted antibody against Smad2/3 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 60 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.
9. Add 60µl of substrate to each well and incubate for 5-10 minutes.
10. Add 30µl 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.