Signosis
Innovative Plate Assay Solutions

Smad1/5/8 ELISA Kit (Chemiluminescence)

Catalog Number TE-0014 **(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 Smad1/5/8 ELISA kit to facilitate the study of BMP/Smad pathway.

.Principle of the assay

Smad ELISA kit is high sensitive and specific assay with a simple and optimized procedure. The 96-well (8X12 strip) white 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 Smad is detected with a specific antibody against Smad2/3 or 1/5/8 and a HRP conjugated secondary antibody. The assay utilizes chemiluminescence detection method, which can be easily measured.

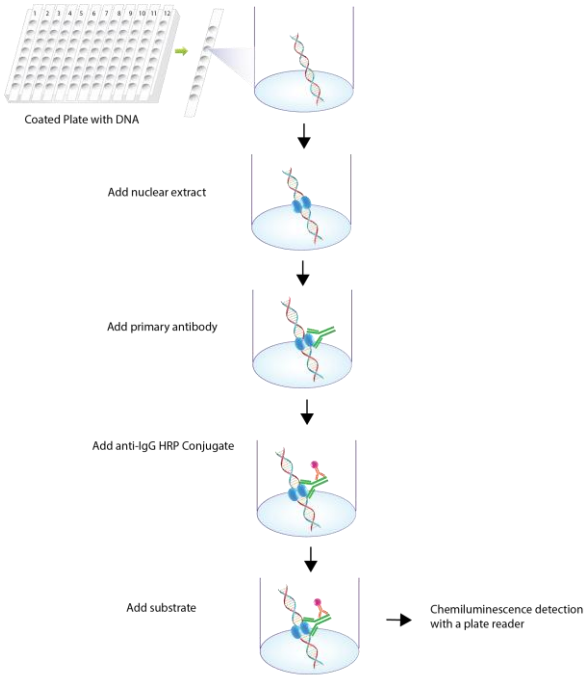

Diagram of TF ELISA

Materials provided with the kit

- 8x12 96-well microplate coated with Smad consensus oligo (4°C).
- Antibody against Smad1/5/8 (4°C).
- HRP conjugate secondary antibody (4°C)
- 2X TF binding buffer (-20°C).
- 1X Nuclear extract dilution buffer (-20°C).
- 5X Assay wash buffer (4°C).
- 1X Diluent buffer (4°C).
- Positive/Negative control Nuclear Extract (-80°C).
- Substrate A (4°C)
- Substrate B (4°C)
- Substrate dilution buffer (4°C)

Material required but not provided

- Luminometer plate reader
- 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 Smad1/5/8 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
   25ul 2X TF binding buffer
   X Nuclear extract (2-10ug)
   X 1X Nuclear extract dilution buffer
   Total 50ul
3. Add the mix on a well and incubate for 1 hour with gently shaking at room temperature.
4. Discard the contents 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 50µl of diluted antibody against Smad1/5/8 to each well and incubate for 2 hour at room temperature with gentle shaking, or 4oC for overnight without shaking.
6. Repeat the aspiration/wash as in step 4.
7. Add 50 µl of diluted HRP conjugate secondary antibody to each well and incubate for 45 min at room temperature with gentle shaking.
8. Invert the plate over an appropriate container and expel the contents forcibly. Wash the plate by adding 200µl of 1x Assay wash buffer. Incubate wash buffer for 10 minutes on a shaker. Repeat washing process two times for a total of three washes with 10 minutes incubation between each wash. Complete removal of liquid at each wash by firmly tapping the plate against a pile of clean paper towels.
   Note: It is important to incubate wash buffer for 10 minutes during each wash to reduce high background in the blank wells.

9. Freshly prepare the substrate solution
   For the whole plate:
   0.6ml Substrate A
   0.6ml Substrate B
   4.8ml Substrate dilution buffer
10. Add 50µl substrate solution to each well.
11. Place the plate in the luminometer, and incubate the plate inside the reading chamber (in dark) for 2 minutes. Set integration time to 1 second with no filter position. For the best results, read the plate within 5-20 minutes.