Signosis
Innovative Plate Assay Solutions

Smad2/3 and 1/5/8 ELISA Kit (Colorimetric)

Catalog Number TE-0015 **(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 byTGF-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 for the analysis of TGF-β/Smad pathway, Smad1/5/8 ELISA kit for the analysis of BMP/Smad pathway, and a combined kit (48 wells forSmad2/3 and 48 wells for Smad1/5/8) to facilitate studying activation of different Smad-related pathways.

Principle of the assay

Smad2/3 and 1/5/8 ELISA kit is high sensitive and specific assay with a simple and optimized procedure. The 96-well (8X12 strip) clear plate is pre-immobilized with the Smad2/3 (6 strips) and Smad1/5/8 (6 strips) 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 Smad1/5/8 subunit and a HRP conjugated secondary antibody. The assay utilizes colorimetric detection method, which can be easily measured by spectrophotometry.

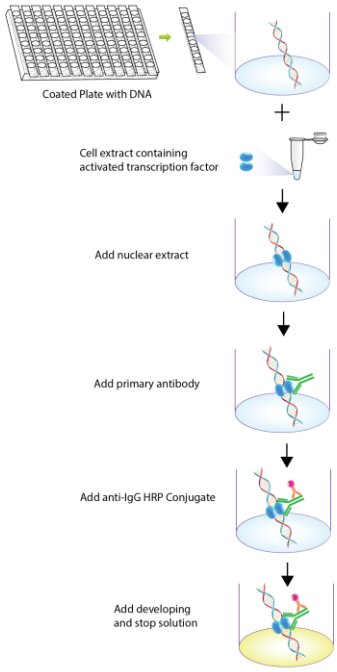


Diagram of TF ELISA

Materials provided with the kit

- 96 well microplate coated with Smad2/3 (6 strips) and Smad1/5/8 (6 strips) consensus oligo (4°C).
- Antibodies against Smad2/3 (-20°C).
- Antibodies against Smad1/5/8 (-20°C).
- HRP conjugate secondary antibody (4°C)
- 2X TF binding buffer (-20°C).
- 1X Nuclear extract dilution buffer (-20°C).
- Smad2/3 positive control (-20°C)
- Smad1/5/8 positive control (-20°C)
- HRP conjugate secondary antibody (4°C)
- 1X Diluent buffer (4°C)
- 5X Assay wash buffer (4°C)
- Substrate (4°C)
- Stop Solution (4°C)

Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

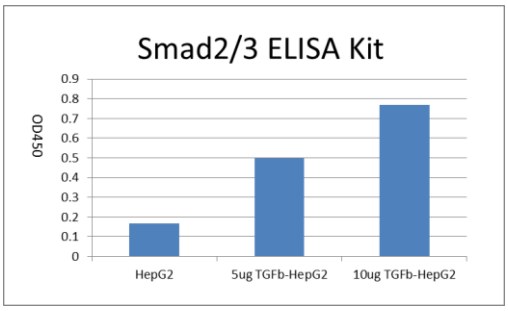
Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute 250 times of antibody against Smad2/3 and Smad1/5/8 with 1X Diluent buffer before use.
- Dilute 1000 times of HRP conjugate secondary antibody with 1X Diluent buffer before use.

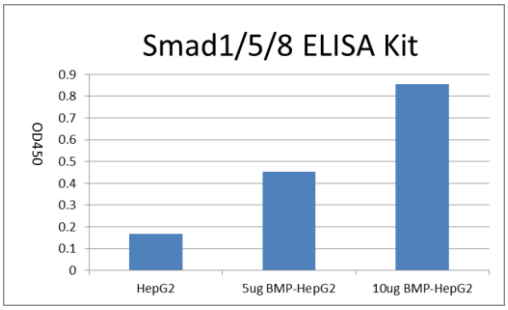
Assay procedure

1. Cut the sealing film over the plate and remove it from the desired number of well strips. Make sure the rest of wells are well sealed.
2. Make TF binding mix
   25ul 2X TF binding buffer
   X Nuclear extract (2-10ug)
   X Nuclear extract dilution buffer
   Total 50ul
3. Add the mix on a well and incubate for 1 hour without shaking.
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 100µl of diluted antibody against Smad2/3 or Smad1/5/8 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 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.
9. Add 100µl of substrate to each well and incubate for 5-10 minutes.
10. Add 50µl 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.

Example of standard curve



Smad2/3 ELISA kit analysis of TGFb/Smad pathway. The HepG2 cells were treated with or without 10ng TGFb for 5 hours, and the nuclear extract were prepared and subject to ELISA kit.



Smad1/5/8 ELISA kit analysis of TGFb/Smad pathway. The HepG2 cells were treated with or without BMP for 5 hours, and the nuclear extract were prepared and subject to ELISA kit.

Diagram for Smad2/3 and 1/5/8 TF-ELISA Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8
B	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8
C	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8
D	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8
E	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8
F	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8
G	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8
H	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad2/3	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8	Smad1/5/8