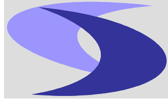

Signosis
Innovative Plate Assay Solutions

IRF7 ELISA Kit

Catalog Number TE-0006 **(For Research Use Only)**

Introduction

IRF3 and IRF7 are members of the interferon regulatory transcription factor (IRF) family, involving in antiviral defense, cell growth regulation, and immune activation. Latent cytoplasmic IRF-3 and IRF7 are activated and phosphorylated following virus infection or treatment with dsRNA, and translocate to the nucleus and bind to their DNA binding sequences. Even though IRF3 and IRF7 are the key activators of the alpha/beta IFN genes, the recent studies have demonstrated that IRF3 and IRF7 have distinct DNA binding properties and induce preferentially on different promoters. Because of the common and distinct biological features of IRF-3 and IRF7, Signosis have developed both IRF3 and IRF7 ELISA kits respectively and a combined kit with specific DNA binding sites and antibodies to distinguish the activation of IRF3 and IRF7 in various biological conditions.

Principle of the assay

IRF7 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 IRF7 consensus sequence oligos. The activated IRF7 in nuclear extract or the whole cell lysate is added in the well and binds to the oligos. The activated IRF7 is detected with a specific antibody against IRF7 subunit and a HRP conjugated secondary antibody. The assay utilizes colorimetric detection method, which can be easily measured by spectrophotometry.

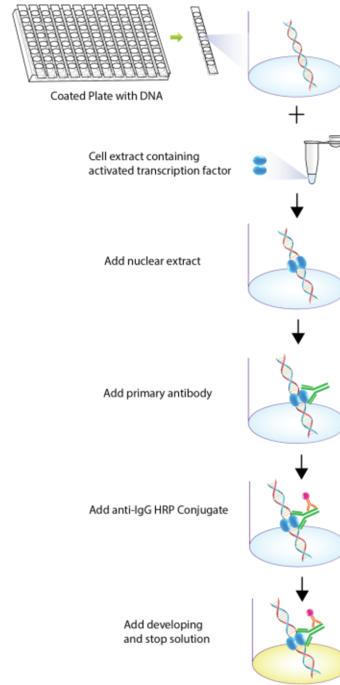

Diagram of TF ELISA

Materials provided with the kit

- 8x12 96-well microplate coated with IRF7 consensus oligo (4°C).
- Antibody against IRF7 (4°C).
- HRP conjugate secondary antibody (4°C)
- 2X TF binding buffer (-20°C)
- 1X Nuclear extract dilution buffer (4°C)
- 1X Diluent buffer (4°C)
- 5X Assay wash buffer (4°C)
- IRF7 Positive control (-80°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
  40 ml 5x Assay wash buffer
  160 ml ddH2O
- Dilute 200 times of antibody against IRF7 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
   30 ul 2X TF binding buffer
   X Nuclear extract (2-10 ug)
   X Nuclear extract dilution buffer
   Total 60 ul
   For the positive control, add 2 ul positive control nuclear extract provided and fill with TF binding buffer and Nuclear extract dilution buffer up to 60 ul.
3. Discard the contents and wash by adding 200 µl 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.
4. Add 60 µl of diluted antibody against IRF7 to each well and incubate for 2 hours at room temperature with gentle shaking, or 4°C overnight without shaking.
5. Repeat the aspiration/wash as in step 4.
6. Add 60 µl of diluted anti-mouse IgG HRP conjugate secondary antibody to each well and incubate for 45 minutes at room temperature with gentle shaking (DO NOT incubate longer than 45 mins to prevent from high background)
7. Repeat the aspiration/wash as in step 4.
8. Add 60µl of substrate to each well and incubate for 5-15 minutes or until positive wells turn blue.
9. Add 30 µl 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 immediately.