Signosis
Innovative Plate Assay Solutions

IRF3 ELISA Kit (Colorimetric)

Catalog Number TE-0005 **(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 IRF-7, 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

The IRF3 ELISA kit is a highly sensitive and specific assay with a simple and optimized procedure. The 96-well (8X12 strip) clear plate is pre-immobilized with the IRF3 consensus sequencing oligo. The activated IRF3 in nuclear extract or the whole cell lysate is added in the well and binds to the oligo. The activated IRF3 is detected with a specific antibody against the IRF3 subunit and a HRP conjugated secondary antibody. The assay utilizes a colorimetric detection method which can be easily measured by spectrophotometry.

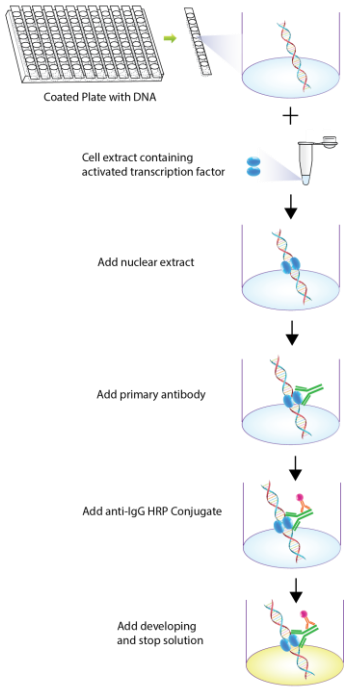

Diagram of TF ELISA

Materials provided with the kit

- 8x12 96-well microplate coated with IRF3 consensus oligo (4ºC).
- Antibody against IRF3 (4ºC).
- HRP conjugate secondary antibody (4ºC)
- 2X TF binding buffer (-20ºC).
- Nuclear extract dilution buffer (4ºC).
- IRF3 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
  40 ml 5x Assay wash buffer
  160 ml ddH2O
- Dilute 100 times of antibody against IRF3 with 1X Diluent buffer before use.
- Dilute 500 times of HRP conjugate secondary antibody with 1X Diluent buffer before use.
- Avoid contact of Substrate and Stop Solution with any metal surfaces.
- Important Note: Before beginning your experiment, quickly take 50µL of the Substrate and check to make sure it's clear. **If its color has changed to dark blue, do not begin the experiment and contact us immediately.**

Assay procedure

1. Calculate the number of samples to decide how many strips need to be used.
2. Make TF binding mix
   30µl 2X TF binding buffer
   X Nuclear extract (2-10µg)
   X Nuclear extract dilution buffer
   Total 60µl
   For the positive control, add 1µl positive control and 29µl nuclear extract dilution buffer. Add 30µl TF binding buffer to make a total of 60µl.
3. Add the mix on a well and incubate at room temperature for 1-2 hours (or overnight at 4°C) with gentle 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. 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 60µl of diluted antibody against IRF3 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 60µl 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. Before adding substrate, check to make sure it is clear. **If the substrate is already blue, please leave the wash buffer in the wells, seal and store the plate at 4°C, and contact us immediately.**
10. Add 60µl of substrate to each well and incubate. Positive wells will turn blue.
11. Add 30µl of stop solution to each well when the blank wells begin to turn blue. The color in the wells should change from blue to yellow.
12. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.