



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

IRF3 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 IRF consensus sequencing oligo. The activated IRF 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 IRF3 subunit and a HRP conjugated secondary antibody. The assay utilizes colorimetric detection method, which can be easily measured by spectrophotometry.

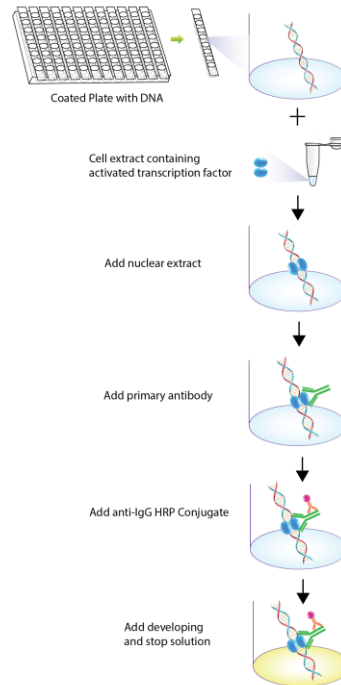


Diagram of TF ELISA

Materials provided with the kit

- 8x12 96-well microplate coated with IRF3 consensus oligo (4°C).
- Antibody against IRF3 (4°C).
- HRP conjugate secondary antibody (4°C)
- 2X TF binding buffer (-20°C).
- Nuclear extract dilution buffer (4°C).
- IRF3 Positive control (-80°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
40 ml 5x Assay wash buffer
160 ml ddH₂O
- Dilute 200 times of antibody against IRF3 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 μ l 2X TF binding buffer
X Nuclear extract (2-10 μ g)
X Nuclear extract dilution buffer
Total 60 μ l
For the positive control, add 2 μ l positive control nuclear extract provided and fill with TF binding buffer and Nuclear extract dilution buffer up to 60 μ l.
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 200 μ l 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 60 μ l 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 60 μ l 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 60 μ l of substrate to each well and incubate. Positive wells will turn blue.
10. Add 30 μ l 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.
11. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.