



IRF3/IRF7 ELISA Kit

Catalog Number TE-0007

(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

IRF3/7 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 IRF3 (6 strips) and IRF7 (6 strips) 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 IRF is detected with a specific antibody against IRF3 or IRF7 subunit and a HRP conjugated secondary antibody. The assay utilizes colorimetric detection method, which can be easily measured by spectrophotometry.

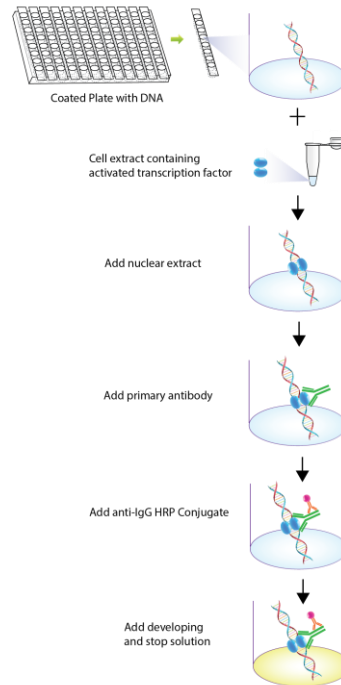


Diagram of TF ELISA

Materials provided with the kit

- 96 well microplate coated with IRF3 (6 strips) and IRF7 (6 strips) consensus oligo (4°C).
- Antibodies against IRF3 and IRF7 (-20°C).
- HRP conjugate secondary antibody (4°C)
- 2X TF binding buffer
- Nuclear extract dilution buffer
- 1X Diluent buffer (4°C)
- 5X Assay wash buffer (RT)
- Poly (I/C) treated 293 nuclear extract control (-20°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.

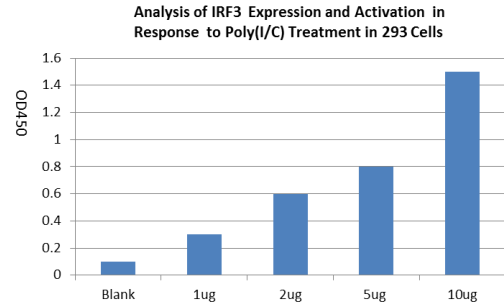
Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
40ml 5x Assay wash buffer
160ml ddH₂O
- Dilute 250 times of antibody against IRF3 and IRF7 with 1X Diluent buffer before use.
- Dilute 500 times of HRP conjugate secondary antibody with 1X Diluent buffer before use.

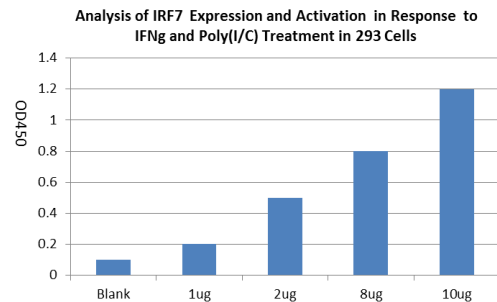
Assay procedure

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 30 minutes 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 IRF3 or IRF7 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



The 293 cells were treated with 20ng/ml poly(I/C) for 8 hours. The nuclear extracts were prepared and subjected to IRF3 ELISA kit.



The 293 cells were treated with 20ng/ml IFN γ and 10ng/ml poly(I/C) for 8 hours. The nuclear extracts were prepared and subjected to IRF7 ELISA kit.