Signosis
Innovative Plate Assay Solutions

ATF4/ATF6 ELISA Kit ELISA Kit (Colorimetric)

Catalog Number TE-0037 **(For Research Use Only)**

Introduction

The unfolded protein response (UPR) is regulated by three ER transmembrane sensors: PERK, IRE1, and ATF6, which activate signaling pathways that regulate gene expression in response to ER stress. ATF4 and ATF6 are two major UPR transcription factors with distinct functions. ATF6 is activated by proteolytic cleavage and translocates to the nucleus, where it regulates genes involved in the ER stress response. ATF4, activated through the PERK-eIF2α pathway, regulates genes involved in amino acid metabolism, antioxidant responses, and apoptosis. Under prolonged or severe ER stress, ATF4 induces the pro-apoptotic protein CHOP. To help distinguish ATF4 and ATF6 activity during ER stress, Signosis has developed an ATF4/ATF6 ELISA kit for detecting and comparing their DNA-binding activities in human, mouse, and rat samples.

.Principle of the assay

ATF4/ATF6 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 ATF4 (6 strips) and ATF6 (6 strips) consensus sequencing oligo. The activated TF in nuclear extract or the whole cell lysate is added in the well and binds to the oligo. The activated ATF4 and ATF6 are detected with a specific antibody against ATF4 or ATF6 subunit and a HRP conjugated secondary antibody. The assay utilizes colorimetric detection method, which can be easily measured by spectrophotometry.

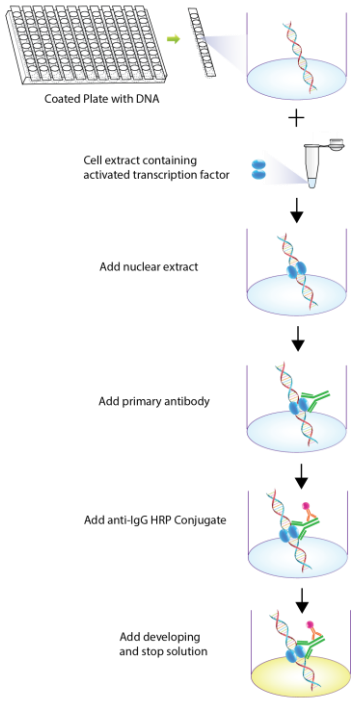


Diagram of TF ELISA

Materials provided with the kit

- 8x12 96-well microplate coated with ATF4/ATF6 consensus oligo (4ºC)
- Antibody against ATF4 (4ºC)
- Antibody against ATF6 (4ºC)
- HRP conjugate secondary antibody (4ºC)
- 2X TF binding buffer (-20ºC)
- 1X Nuclear extract dilution buffer (-20ºC)
- ATF4 positive control (-80ºC)
- ATF6 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
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute 100 times of antibody against ATF4 or ATF6 with 1X Diluent buffer before use.
- Dilute 1000 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.**

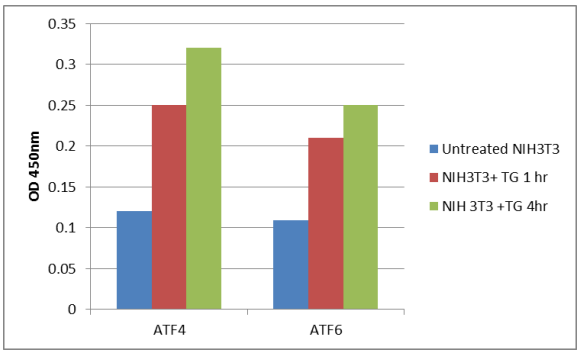
Assay procedure

1. Calculate the number of samples to decide how many strips need to be used.
2. Make TF binding mix

   30ul 2X TF binding buffer
   X Nuclear extract (2-10µg)
   X µl Nuclear extract dilution buffer
   Total 60µl

   For the positive control, add 20µl positive control, and 10µl nuclear extract dilution buffer. Add 30µl TF binding buffer to make a total of 60µl.
3. Add 60µl of sample or positive control to the wells and incubate for 1-2 hours at room temperature with gentle shaking.
4. Discard the contents and wash by adding 200µl 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.
5. Add 60µl of diluted antibody against ATF4 or ATF6 to each well and incubate for 2 hours at room temperature with gentle shaking, or 4ºC overnight without shaking.
6. Repeat the aspiration/wash as in step 4.
7. Add 60µl 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 high background)
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 for 5-15 minutes or until negative control wells begin to turn faint blue.
11. Add 30µl of stop solution to each well. 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 immediately.

Example of Analysis Data



Monitoring ATF4 and ATF6 activation with ATF4/AFT6 TF ELISA kit

Nuclear extracts from untreated NIH3T3 cells and NIH 3T3 cells stimulated with Thapsigargin (TG) 150ng/ml for one hour and 4 hours were assayed using ATF4/ATF6 TF ELISA kit.