Signosis
Innovative Plate Assay Solutions

ATF6 ELISA Kit (Colorimetric)

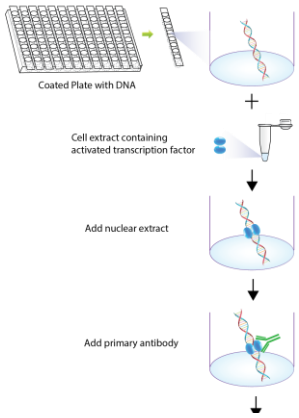
Catalog Number TE-0041 **(For Research Use Only)**

Introduction

Unfolded protein response (UPR) is governed by three transmembrane sensors: PERK, IRE1 and ATF6. These stress sensors control a complex ER-to-nucleus signaling pathway that transmits information across the ER membrane to an extensive gene-expression program. Two major transcription factors for UPR are ATF4 and ATF6. Both ATF4 and ATF6 belong to a basic leucine zipper ATF/CREB family, but have distinct functions. ATF6 is a type II transmembrane ER protein that is activated by proteolytic cleavage. Active ATF6 translocates to the nucleus where it forms active homodimers or dimerizes with NF-Y and XBP1s and binds to promoter regions containing ATF/cAMP response elements (CREs) and/or ER Stress Elements (ERSE). ATF4 belongs to PERK-eIF2-ATF4 axis and regulates the expression of genes involved in amino acid biosynthesis and transport functions, antioxidant stress responses, and apoptosis. ATF4 induces both pro-survival (early) and pro-apoptotic (late) transcriptional programs. Prolonged or extreme ER stress uses ATF4 to upregulate proapoptotic protein C/EBP homologous protein (CHOP). To distinguish the function of ATF4 and ATF6 in ER stress, Signosis has developed ATF4 /ATF6 ELISA kit for detecting and comparing ATF4 and ATF6 DNA binding activities for human, mouse and rat samples.

Principle of the assay

Our 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 ATF6 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 ATF6 are detected with a specific antibody against the ATF6 subunit and a HRP conjugated secondary antibody. The assay utilizes colorimetric detection method, which can be easily measured by spectrophotometry.



Materials provided with the kit

- 8x12 96-well microplate coated with ATF6 consensus oligo (4ºC).
- Antibody against ATF6 (4ºC).
- 2X TF binding buffer (-20ºC).
- 1X Nuclear extract dilution buffer (-20ºC).
- ATF6 positive control (-80ºC)
- Anti-mouse HRP conjugate secondary antibody (4ºC)
- 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 ATF6 with 1x Diluent buffer before use.
- Dilute 500 times of HRP conjugate secondary antibody for ATF6 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
   30ul 2x TF binding buffer
   X Nuclear extract (2-10ug)
   X Nuclear extract dilution buffer
   Total 60ul
   For the positive control, add 20ul positive control provided.
3. Add the mix in the well and incubate at room temperature for 1-2 hours.
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 60µl of diluted antibody against ATF6 to each well and incubate for 2 hours at room temperature with gentle shaking, or 4^{o}C overnight without shaking.
6. Repeat the aspiration/wash as in step 4.
7. Add 60 µl of diluted HRP conjugate secondary antibody for ATF6 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 60µl of substrate to each well and incubate for 5-10 minutes.
10. Add 30µl 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 min.