Signosis
Innovative Plate Assay Solutions

TFEB ELISA Kit

Catalog Number TE-0029 **(For Research Use Only)**

Introduction

TFEB is a member of the basic Helix-Loop-Helix-Zipper family of transcription factors that specifically recognizes and binds E-box sequences (5'-CANNTG-3') on DNA. TFEB is a master gene for lysosomal biogenesis, and therefore plays a central role in regulating expression of lysosomal genes. TFEB also acts as a positive regulator of autophagy by promoting expression of genes involved in autophagy. Under aberrant lysosomal storage conditions such as in lysosomal storage diseases, TFEB is translocated from the cytoplasm to the nucleus, resulting in the activation of its target genes. Signosis has developed the TFEB ELISA kits for sensitive and specific analysis of the activities of TFEB in a high throughput manner. The kit can be used for human, mouse and rat samples.

Principle of the assay

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

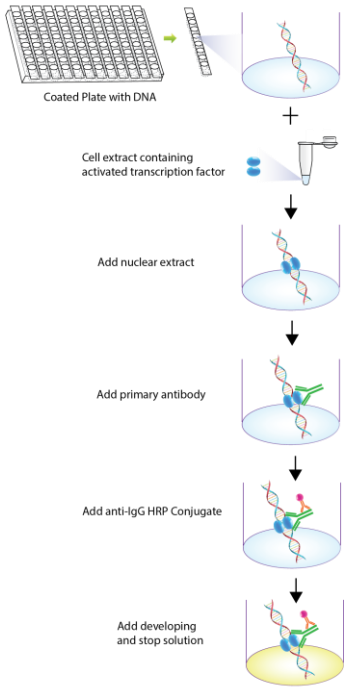


Diagram of TF ELISA

Materials provided with the kit

- 8x12 96-well microplate coated with TFEB consensus oligo (4ºC)
- Antibody against TFEB (4ºC)
- TFEB positive control nuclear extract (-20ºC)
- Positive control (-80ºC).
- HRP conjugate secondary antibody (4ºC)
- 2X TF binding buffer (-20ºC)
- 1X Nuclear extract dilution buffer (-20ºC)
- 1X Diluent buffer (4ºC)
- 5X Assay wash buffer (RT)
- Substrate (4ºC)
- Stop Solution (4ºC)

Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

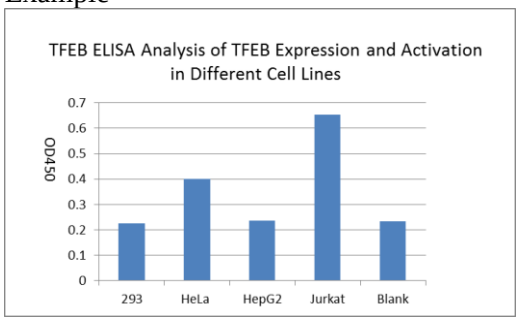
Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute 100 times of antibody against TFEB with 1X Diluent buffer before use.
- Dilute 500 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
   30ul 2X TF binding buffer
   X Nuclear extract (2-10ug)
   X Nuclear extract dilution buffer
   Total 60ul
   For the positive control, add 30ul positive control nuclear extract provided.
3. Add the mix on a well and incubate for 1 hour 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 60µl of diluted antibody against TFEB 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 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.
   On the last wash, incubate 1X Assay wash buffer in wells for 10 minutes on a shaker. This step is important for reducing background in blank wells.
9. Add 60µl of substrate to each well and incubate. Positive wells will turn blue.
10. 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.
11. Determine the optical density of each well with a microplate reader at 450 nm within 30 minutes.

Example



Nuclear extracts were prepared from different cells and subjected to TFEB ELISA assay.