Signosis
Innovative Plate Assay Solutions

NRF2 ELISA Kit (Colorimetric)

Catalog Number TE-0027 **(For Research Use Only)**

Introduction

NRF2 plays a crucial role in cellular anti-oxidant defense, making it a therapeutic target for neurodegenerative diseases and cancer. Under unstressed conditions, NRF2 is kept in the cytoplasm by a cluster of proteins that degrade it quickly. Under oxidative stress, NRF2 is not degraded but instead translocates to the nucleus where it binds to a DNA promoter and initiates gene expression. In the nucleus, NRF2 forms a heterodimer with a small Maf protein and binds to the Antioxidant Response Element in the upstream promoter region of many antioxidative genes and initiates their transcription. Signosis has developed the NRF2 ELISA kit for the analysis of the NRF2 pathway and to facilitate studying activation of different NRF2-related pathways.

Principle of the assay

The NRF2 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 NRF2 consensus sequencing oligo. When your nuclear extract or whole cell lysate is added to the well, activated NRF2 binds to the oligo. The activated NRF2 is detected with a specific antibody against an NRF2 subunit and a HRP conjugated secondary antibody. The assay utilizes colorimetric detection, which can be easily measured by spectrophotometry.

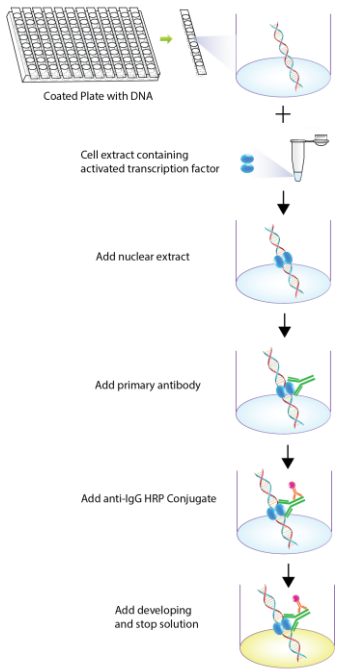

Diagram of TF ELISA

Materials provided with the kit

- 8x12 96-well microplate coated with NRF2 consensus oligo (4ºC).
- Antibody against NRF2 (4ºC).
- Anti Mouse IgG HRP conjugate secondary antibody (4ºC)
- 2X TF binding buffer (-20ºC).
- 1X Nuclear extract dilution buffer (-20ºC).
- NRF2 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
  40 ml 5X Assay wash buffer
  160 ml ddH2O
- Dilute 100 times of antibody against NRF2 with 1X Diluent buffer before use.
- Dilute 500 times of HRP conjugate secondary antibody with 1X Diluent buffer before use.

Assay procedure

Step 1: Binding of NRF2 to its Consensus Sequence

1. Calculate the number of samples to decide how many strips need to be used.

2. Make TF binding mix

   30 ul 2X TF binding buffer
   X Nuclear extract (10-20ug)
   X ul Nuclear Extract Dilution Buffer
   Total 60ul

   For the positive control, add 1ul positive control, and 29ul Nuclear Extract Dilution Buffer.
   Add the mix in a well and incubate with mild agitation on a rocking platform at room temperature for 1-2 hours.

3. Discard the contents and wash by adding 200µl of 1X Assay wash buffer. Repeat the process for a total of three washes. Completely remove the liquid at each wash. After the last wash, remove any remaining liquid by inverting the plate against clean paper towels.

Step 2: Binding of Primary Antibody

4. Add 60 µl of diluted antibody against NRF2 to each well and use the provided adhesive cover to seal the plate. Incubate overnight at 4ºC without agitation.
5. Repeat the aspiration/wash steps in Step 1. No,3

Step 3: Binding of Secondary Antibody

6. Add 60 µl of diluted anti-mouse IgG HRP conjugate secondary antibody to each well and incubate for 45 minutes at room temperature without agitation.
7. Repeat the aspiration/wash steps in Step 1. No,3

Step 4: Colorimetric Reaction

8. Add 60 µl of substrate to each well and incubate for 5-15 minutes or until positive wells turn blue.
9. Add 30 µl of stop solution to each well. The color in the wells should change from blue to yellow.
10. Determine the optical density of each well with a microplate reader at 450 nm immediately.