



## 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 high 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. The activated NRF2 in nuclear extract or the whole cell lysate is added in the well and 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 method, which can be easily measured by spectrophotometry.

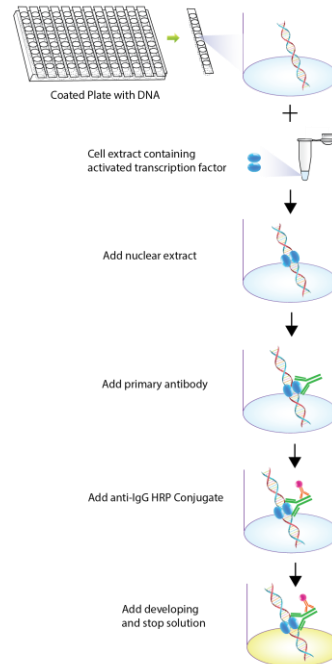


Diagram of TF ELISA

### Materials provided with the kit

- 8x12 96-well microplate coated with NRF2 consensus oligo (4°C).
- Antibody against NRF2 (-20°C).
- HRP conjugate secondary antibody (4°C)
- 2X TF binding buffer (-20°C).
- 1X Nuclear extract dilution buffer (-20°C).
- NRF2 positive control (-20°C)
- 1X Diluent buffer (4°C)
- 5X Assay wash buffer (4°C)
- Substrate (4°C)
- Stop Solution (4°C)

### Material required but not provided

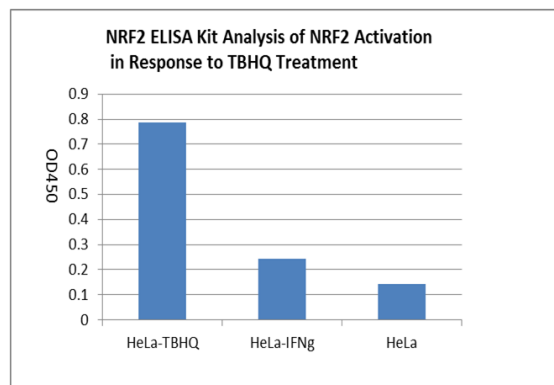
- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

## Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer  
40ml 5x Assay wash buffer  
160ml ddH<sub>2</sub>O
- 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

1. Calculate the number of samples to decide how many strips need to be used.
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 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 100µl of diluted antibody against NRF2 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 10-30 minutes or until positive wells turn blue.
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.



Analysis of NRF2 activation with NRF2 ELISA in TBHQ-Treated HeLa Cells.

The HeLa cells were treated with 50uM TBHQ overnight. The nuclear extracts were prepared and subjected to NRF2 ELISA kit.