Signosis
Innovative Plate Assay Solutions

NFkB p50 ELISA Kit

Catalog Number TE-0002 **(For Research Use Only)**

Introduction

NF-kappaB (NFkB) proteins comprise a family of eukaryotic transcription factors that are involved in the control of a large number of cellular and organismal processes. In addition, these transcription factors are associated with many diseases including cancer and arthritis. NFkB commonly refers specifically to a p50-RelA(p65) heterodimer, which is the major Rel/NF-kB complex in most cells. P65-p65 and p50-p50 heterodimers have been demonstrated to bind on DNA as well. NF-kB is present as a latent, inactive, IkB-bound complex in the cytoplasm. When a cell receives any of a multitude of extracellular signals, NF-kB rapidly enters the nucleus and activates gene expression. Signosis developed the NFkB-p65 and NFkB p50 ELISA kits for sensitive and specific analysis of the activities of NFkB in a high throughput way. The kit can be used for human, mouse and rat samples.

.Principle of the assay

NFkB p50 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 NFkB consensus sequencing oligo. The activated NFkB in nuclear extract or the whole cell lysate is added in the well and binds to the oligo. The activated NFkB is detected with a specific antibody against p50 subunit and a HRP conjugated secondary antibody. The assay utilizes colorimetric detection method, which can be easily measured by spectrophotometry.

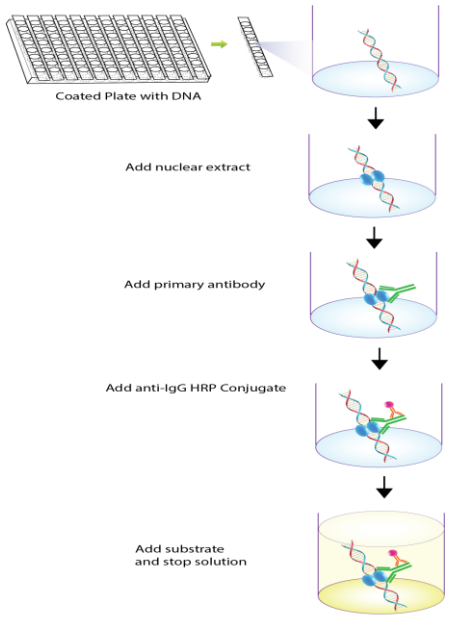


Diagram of TF ELISA

Materials provided with the kit

- 96 well microplate coated with NFkB consensus oligo (4°C).
- Antibody against NFkB p50 (-20°C).
- HRP conjugate secondary antibody (4°C)
- 2X TF binding buffer
- Nuclear extract dilution buffer
- 1X Diluent buffer (4°C)
- 5X Assay wash buffer (RT)
- HeLa-TNF Positive control (-20°C)
- Competition oligo (-20°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.

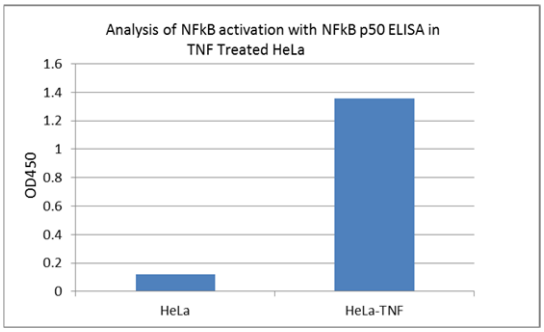
Reagent preparation before starting experiment

- Dilute the 5x Assay wash buffer to 1x buffer
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute 500 times of antibody against NFkB p50 with 1X Diluent buffer before use.
- Dilute 1000 times of HRP conjugate secondary antibody with 1X Diluent buffer before use.

Assay procedure

1. Cut the sealing film over the plate and remove it from the desired number of well strips. Make sure the rest of wells are well sealed.
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 30 minutes 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 NFkB p50 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 5-10 minutes.
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.

Example of standard curve



Analysis of NFkB activation with NFkB p50 ELISA in TNF-Treated HeLa Cells.
2ug HeLa and HeLa-TNF treated nuclear extracts are subjected to analyze with NFkB p50 ELISA kit.