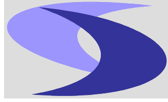


Signosis
Innovative Plate Assay Solutions

NFkB p65 ELISA Kit (Colorimetric)

Catalog Number TE-0001 **(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 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 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 p65 subunit and a HRP conjugated secondary antibody. The assay utilizes colorimetric detection method, which can be easily measured by spectrophotometry.

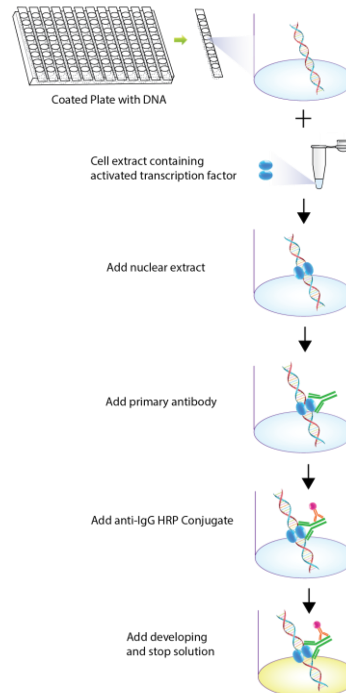


Diagram of TF ELISA

Materials provided with the kit

- 8x12 96-well microplate coated with NFkB consensus oligo (4°C)
- Antibody against NFkB p65 (4°C)
- HRP conjugate secondary antibody (4°C)
- 2X TF binding buffer (-20°C)
- 1X Nuclear extract dilution buffer (-20°C)
- NFkB Positive control (-80°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 ddH2O
- Dilute 100 times of antibody against NFkB p65 with 1X Diluent buffer before use.
- Dilute 1000 times of HRP conjugate secondary antibody with 1X Diluent buffer before use.
- Avoid contact of Substrate and Stop Solution with any metal surfaces.
- Important Note: Before beginning your experiment, quickly take 50µL of the Substrate and check to make sure it's clear. **If its color has changed to dark blue, do not begin the experiment and contact us immediately.**

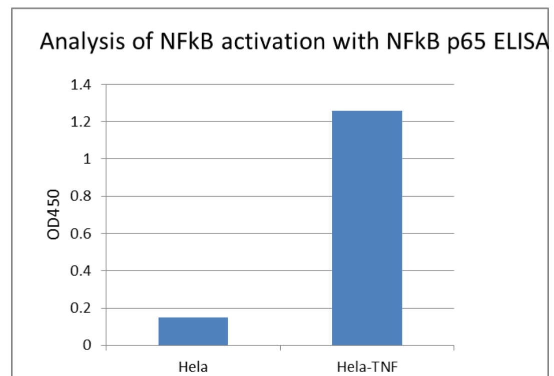
Assay procedure

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 (2-10µg)
   X µl Nuclear extract dilution buffer
   Total 60µl

   For the positive control, add 3µl positive control, and 27µl nuclear extract dilution buffer. Add 30µl TF binding buffer to make a total of 60µl.
3. Add 60µl of sample or positive control to the wells and incubate for 1-2 hours at room temperature with gentle shaking.
4. Discard the contents and wash by adding 200µl of 1X Assay wash buffer. Repeat the process 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 NFkB p65 to each well and incubate for 2 hours at room temperature with gentle shaking, or 4°C overnight without shaking.
6. Repeat the aspiration/wash as in step 4.
7. Add 60µl of diluted anti-mouse IgG HRP conjugate secondary antibody to each well and incubate for 45 minutes at room temperature with gentle shaking (DO NOT incubate longer than 45 mins to prevent high background)
8. Repeat the aspiration/wash as in step 4.
9. Before adding substrate, check to make sure it is clear. **If the substrate is already blue, please leave the wash buffer in the wells, seal and store the plate at 4°C, and contact us immediately.**
10. Add 60µl of substrate to each well and incubate for 5-15 minutes or until negative control wells begin to turn faint blue.
11. Add 30µl of stop solution to each well. The color in the wells should change from blue to yellow.
12. Determine the optical density of each well with a microplate reader at 450 nm immediately.

Example of standard curve



Analysis of NFkB activation with NFkB p65 ELISA in TNF-Treated HeLa Cells.
2ug HeLa and HeLa-TNF treated nuclear extracts are subjected to analyze NFkB p65 ELISA kit.