Signosis
Innovative Plate Assay Solutions

QuantiTF NFkB p65 ELISA Kit (Colorimetric)

Catalog Number QE-0005 **(For Research Use Only)**

Introduction

NF-kappaB (NFkB) proteins comprise a family of eukaryotic transcription factors that are involved in the control of many 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 homodimers have been demonstrated to bind on DNA as well. In the unstimulated cells, 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, NFkB rapidly enters the nucleus and activates gene expression. Signosis developed the QuantiTF NFkB-p65 ELISA kits for sensitive and specific analysis of the activities of NFkB quantitatively in a high throughput way. The kit can be used for human, mouse and rat samples.

Principle of the assay

QuantiTF NFkB 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 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 primary antibody against p65 subunit and a HRP conjugated secondary antibody. The assay utilizes colorimetric detection method, which can be easily measured by spectrophotometry. In the kit, we also provide NFkB recombinant protein standard so that the activity of NFkB in cells can be quantitatively studied.

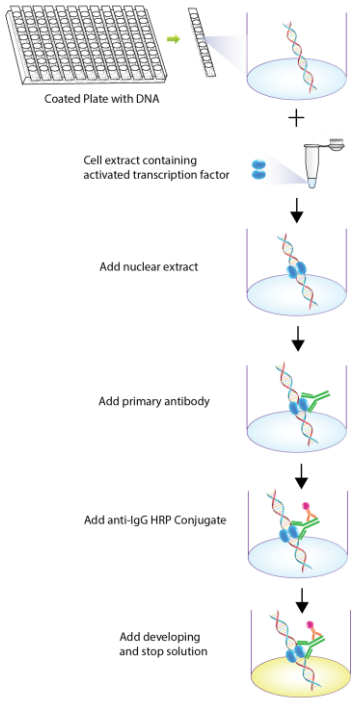


Diagram of TF ELISA

Materials provided with the kit

- 96-well (8x12) microplate coated with NFkB consensus oligo (4ºC)
- Primary antibody against NFkB p65 (4ºC)
- HRP conjugate secondary antibody (4ºC)
- 2X TF binding buffer (-20ºC)
- 1X Nuclear extract dilution buffer (-20ºC)
- NFkB p65 recombinant protein standard (-20ºC)
- 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
  40ml 5x Assay wash buffer
  160ml ddH2O
- Dilute 200 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.

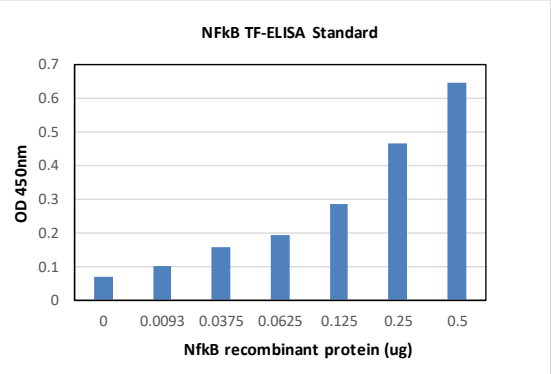
Assay procedure

1. Calculate the number of samples to decide how many strips need to be used.
2. Make TF binding mix as following:

   25ul 2X TF binding buffer
   X Nuclear extract (2-10ug)
   X Nuclear extract dilution buffer
   Total 50ul

   For standard protein serial dilution preparation, please see the instruction in the end of this manual.
3. Add the reaction mix on a well and incubate for 1-2 hours without shaking at room temperature.
4. Discard the solution 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 100µl of diluted antibody against NFkB p65 to each well and incubate for 1.5 hour at room temperature with gentle shaking.
6. Repeat the aspiration/wash as in step 4 for a total of three washes.
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 for a total of three washes.
9. Add 100µl of substrate to each well and incubate for 30 minutes. The solution will gradually change to blue.
10. Add 50µl of stop solution to each well. The solution 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 NFkB standard graph



❖ **Recombinant protein standard for TF-ELISA:**

1. Add appropriate amount of recombinant protein standard to the first well as following:

   50ul 2X TF binding buffer
   Xul Recombinant protein (50ng)
   Xul Nuclear extract dilution buffer
   Total 100ul

2. Add 50ul of Nuclear extract dilution buffer to 2^{nd} -7^{th} wells in the same strips. Leave the 8^{th} as blank.
3. Transfer 50ul solution from the 1^{st} well to the next dilution well.
4. Repeat the transfer until the 7^{th} well (Do not transfer to the 8^{th}). Trash 50ul of the solution from the 7^{th} well.
5. Use the 8^{th} well as blank, adding H_2O instead of protein in the above reaction. Mix and only add 50ul in the 8^{th} well.