CREATIVE BIOMART®
Assay Kit

NF-?B p65 (Human) Transcription Factor Activity Assay Kit

Product Information

Cat.No. Kit-2245

Product Overview

NF-κB is a transcription factor that plays a pivotal role in a number of physiological cell processes such as growth, apoptosis, and immune response through regulating the expression of a number of cellular genes. Normally, NF-κB is inactive and retained in the cytoplasm bound to its inhibitory protein IκB. Upon stimulation of various factors such as infection of pathogens including viruses and bacteria or treatment of cytokines such as IL-1 or TNF-a, IκB proteins are phosphorylated, ubiquitinated and finally degraded. Released NF-κB proteins then form homo- or heterodimers, most commonly p50/p65 or p50/p50, which are transported into the nucleus where they bind to specific DNA sequences of targeted genes and activate transcription. NF-κB also induces expression of its own inhibitor, IκBa, which binds to active NF-κB in the nucleus to terminate the induction of gene expression. The NF-κB p65 Transcription Factor-Activity Assay kit is a non-radioactive transcription factor assay with an ELISA format. It offers an easy, speedy, sensitive and high-throughput method to detect the activation of transcription factors. In 96-well plates, double stranded oligonucleotides containing NF-κ B binding sequence have been coated. These oligonucleotides specifically capture the active NF-κB p65 contained in whole cell lysate or nuclear extracts after a short incubation. The specificity of the reaction between active NF-κB p65 and the DNA probe is additionally stringent because of the establishment of specific competitive DNA and non-specific competitive DNA probes in this reaction system.

Storage

Upon receipt, the positive control should be removed and stored at -20° or -80°C. The remainder of the kit can be stored for up to 6 months at 2-8°C from the date of shipment. Opened Microplate Wells or reagents may be stored for up to 1 month at 2-8°C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge.
Note: The kit can be used within one year if the whole kit is stored at -20°C upon receipt. Avoid repeated freeze-thaw cycles.

Shipping

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Dry ice

Size

100 assays

Kit Components

NF-κB p65 DNA Probe Microplate, 96 wells (12 strips X 8 wells) coated with NF-κB probes, 1 plate
DNA Binding Buffer, 5X concentrated Buffer, 4 ml
Positive Control, Cell nuclear extracts, 1 vial (20 µl)
Specific Competitor DNA Probe, Free DNA probes that compete directly with the coated NF-κB probes. Can bind activated NF-κB, 1 vial
Non-specific Competitor DNA Probe, Free DNA probes with mutations of the coated DNA probe. Cannot bind activated NF-κB, 1 vial
Assay Reagent, 1X solution, 1 vial (200 µl)
DTT, 300 mM DTT, 1 vial (200 µl)
Wash Buffer Concentrate (20X), 20X concentrated solution, 25 ml
NF-κB p65 Primary Antibody, Anti-NF-κB p65 antibody, 1 vial
HRP-conjugated Secondary Antibody, Anti-IgG HRP conjugated antibody, 1 vial
Antibody Diluent Buffer, Buffer solution for diluting primary and secondary antibodies, 25 ml
TMB One-Step Substrate Reagent, 3,3,5,5'-tetramethylbenzidine (TMB) in buffer solution, 12 ml
Stop Solution, 0.2 M sulfuric acid, 8 ml

Materials Required but Not Supplied

1 Microplate reader capable of measuring absorbance at 450 nm.
2 Precision pipettes to deliver 1 ul to 1 ml volumes.
3 Adjustable 1-25 ml pipettes for reagent preparation.
4 100 ml and 1 liter graduated cylinders.
5 Absorbent paper.
6 Distilled or deionized water.
7 Tubes to prepare positive or sample mixtures.

Preparation

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1. Preparation of samples:
Prepare nuclear extraction or whole lysate containing targeted protein NF-κB p65 from cell culture or tissue. We recommend using the RayBiotech Nuclear Extraction Kit to isolate nuclear proteins for subsequent use in this transcription factor activity assay.
2. Preparation of transcription factor binding reaction system: Bring all reagents to room temperature (18 - 25°C) before use. Thaw the positive control and samples and keep them on ice before adding into wells. Prepare 100 ul transcription factor binding reaction system for each well with 5 x TF-Activity Assay DNA Binding Buffer, ATF-ELISA assay Reagent, DTT, Specific Competitor DNA Probe, Non-specific Competitor DNA Probe, and Positive Control or samples containing targeted proteins. Typical examples are shown in the table below.
Note: Each reaction may be prepared in a labeled microfuge tube or directly in the coated plate well. If the reaction system is prepared directly in the coated plate wells, please add the reagents sequentially as shown in the table to get the best results.
COMPONENT Positive control Sample Specific competitor Non-Specific competitor Blank
5x TF-Activity Assay DNA Binding Buffer 20 ul 20 ul 20 ul 20 ul 20 ul
TF-Activity Assay Reagent 1.5 ul 1.5 ul 1.5 ul 1.5 ul 1.5 ul
DTT 1 ul 1 ul 1 ul 1 ul 1 ul
Specific Competitor - - 10 ul - -
Non-specific Competitor - - - 10 ul -
Control/Sample containing proteins 5 ul * ul * ul * ul -
Total volume bring final volume to 100ul with deionized water
* Please note that the amount of total protein containing the target protein to be used in this test can be optimized and must be determined by the investigator.
3. Preparation of primary antibody:
Briefly spin down the TF-Activity Assay NF-κB p65 Primary Antibody vial. Add 100 ul of Antibody Diluent Buffer into the vial to prepare a primary antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The primary antibody concentrate should then be diluted 100- fold with the Antibody Diluent Buffer and used in step 4 of Part VII Assay Procedure.
4. Preparation of secondary antibody: Briefly spin down the TF-Activity Assay HRP-conjugated

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Secondary Antibody vial before use. Add 100 ul of Antibody Diluent Buffer into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can be stored at 4°C for 5 days). The detection antibody concentrate should then be diluted 100-fold with the Antibody Diluent Buffer and used in step 6 of Part VII Assay Procedure.

5. Preparation of 1x Wash Buffer:

Dilute 25 ml of the 20x Wash Buffer Concentrate into deionized or distilled water to yield 500 ml of 1x Wash Buffer. If the Wash Buffer Concentrate (20x) contains visible crystals, warm to room temperature and mix gently until dissolved.

Note: All reagents containing protein (positive control, samples) should be kept on ice to maintain protein stability.

If the reaction system is prepared directly in the coated plate wells, please add the reagents sequentially as shown in the table to get the best results.

To observe the specificity of the DNA binding activity, the amount of protein used in wells of sample, specific competitor and non-specific competitor must be the same.

A positive control should be included every time to confirm correct operation of experiment, however it is not necessary to run specific competitor and non-specific competitor for each sample and every time.

Assay Protocol

1. Bring the 96-well plate to room temperature (18 - 25°C) before use. If the whole plate will not be used in this assay, place remaining wells back to 2 to 8°C or -20°C. It is recommended that all positive control and samples be run at least in duplicate.

2. Add 100 ul of each prepared transcription factor binding reaction system (see Reagent Preparation step 2) including positive control, specific competitor, non-specific competitor and sample into appropriate wells. Cover wells and incubate for 2 hours at room temperature or overnight at 4°C with gentle shaking.

3. Discard the solution and wash 4 times by filling each well with 300 ul of 1x Wash Buffer (Reagent Preparation step 5) using a multi-channel pipette or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 100 ul of prepared TF-Activity Assay NF-κB p65 Primary Antibody (Reagent Preparation step 3)

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to each well. Incubate for 1 hour at room temperature with gentle shaking.
5. Discard the solution. Repeat the wash as in step 3.
6. Add 100 ul of prepared TF-Activity Assay HRP-conjugated Secondary Antibody (see Reagent Preparation step 4) to each well. Incubate for 1 hour at room temperature with gentle shaking.
7. Discard the solution. Wash as directed in step 3.
8. Add 100 ul of TMB One-Step Substrate Reagent (Item H) to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.
9. Add 50 ul of Stop Solution (Item I) to each well. Read at 450 nm immediately.

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