CREATIVE BIOMART®
Assay Kit

NF-kB (p65) Transcription Factor Assay Kit

Product Information

Cat.No. Kit-0622

Product Overview

NF-κB (p65) Transcription Factor Assay is a non-radioactive, sensitive method for detecting specific NF-κB (p65) DNA binding activity in nuclear extracts. It replaces the cumbersome radioactive electrophoretic mobility shift assay (EMSA) with an ELISA. This kit is an ideal way to measure NF-κB transcriptional activity downstream of drug treatment or manipulation of cells in vitro or in vivo. NF-κB (p65) Transcription Factor Assay detects human, mouse, and rat NF-κB (p65). It does not cross react with NF-κB (p50).

Storage

Kit components may be stored at -20°C prior to use. For long-term storage, the Transcription Factor NF-κB (human p65) Positive Control should be thawed on ice, aliquoted at 25 µl/vial, and stored at -80°C.

Shipping

Dry ice

Size

96 wells

Kit Components

Transcription Factor Binding Assay Buffer (4X), 1 vial/3 ml, 4°C
Transcription Factor Reagent A, 1 vial/120 µl, -20°C
Transcription Factor NF-κB (human p65) Positive Control, 1 vial/150 µl, -80°C
Transcription Factor Antibody Binding Buffer (10X), 1 vial/3 ml, 4°C
Transcription Factor NF-κB (p65) Primary Antibody, 1 vial/120 µl, -20°C
Wash Buffer Concentrate (400X), 1 vial/5 ml, RT
Polysorbate 20, 1 vial/3 ml, RT
Transcription Factor NF-κB Competitor dsDNA, 1 vial/120 µl, -20°C
Transcription Factor Goat Anti-Rabbit HRP Conjugate, 1 vial/120 µl, -20°C

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Transcription Factor NF-κB 96-Well Strip Plate, 1 plate, 4°C
96-Well Cover Sheet, 1 cover, RT
Transcription Factor Developing Solution, 1 vial/12 ml, 4°C
Transcription Factor Stop Solution, 1 vial/12 ml, RT

Materials Required but Not Supplied

1. A plate reader capable of measuring absorbance at 450 nm
2. A source of pure water; glass Milli-Q or HPLC-grade water is acceptable
3. 300 mM Dithiothreitol (DTT)
4. Nuclear Extraction Kit or buffers for preparation of nuclear extracts

NOTE: The components in each kit lot have been quality assured and warranted in this specific combination only; please do not mix them with components from other lots.

Preparation

1. Transcription Factor Antibody Binding Buffer (1X) Preparation

Dilute the Transcription Factor Antibody Binding Buffer (10X) 1:10 by adding 27 ml of UltraPure water. Store at 4°C for up to six months.

2. Wash Buffer (1X) Preparation

Dilute the Wash Buffer (400X) to a total volume of 2 liters with UltraPure water and add 1 ml of Polysorbate 20. Scale as necessary. NOTE: Polysorbate 20 is a viscous liquid and cannot be measured by a pipette. A positive displacement device such as a syringe should be used to deliver small quantities accurately. Store at 4°C for up to two months.

3. Complete Transcription Factor Binding Assay Buffer Preparation

Prepare 10 ml of Complete Transcription Factor Binding Assay Buffer (CTFB) by adding 2.5 ml of Transcription Factor Binding Assay Buffer (4X), 0.1 ml of Transcription Factor Reagent A, and 0.1 ml of 300 mM DTT to 7.3 ml of UltraPure water. Scale as necessary. It is recommended that the CTFB be used the same day it is prepared.

4. Transcription Factor NF-κB (human p65) Positive Control Preparation

Transcription Factor NF-κB (human p65) Positive Control contains 150 µl of clarified cell lysate. This lysate is provided as a positive control (PC) for NF-κB (p65) activation; it is not intended to be used as a standard for quantitative measurements. The positive control provided will produce a strong

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signal (>0.5 AU at 450 nm) when used at 10 µl/well. Serial two-fold dilutions of this PC can be used for monitoring the dynamic range of the assay. A decrease in signal may occur with repeated freeze/thaw cycles. It is recommended that the Transcription Factor NF-κB (human p65) Positive Control be aliquoted at 50 µl per vial and stored at -80°C to avoid loss in signal from repeated freeze/thaw cycles.

Assay Protocol

Positive Control Dilution Set Up

To prepare the PC for use in the ELISA: Obtain six clean test tubes and label them #PC1-PC6. Dilute 45 µl of Transcription Factor NF-κB (human p65) Positive Control with 405 µl of CTFB. This dilution is positive control 1 (PC1). Add 220 µl of CTFB to the tubes that correspond to PC2-PC6. Transfer 220 µl of the PC1 to tube PC2 and mix gently. Transfer 220 µl from PC2 to PC3 and mix gently. Repeat this process for the remaining tubes.

Plate Set Up

There is no specific pattern for using the wells on the plate. A typical layout for the PC1-PC6 serial dilutions and unknown samples of nuclear extracts (S1-S40) to be measured in duplicate is given below. We suggest you record the contents of each well on the template sheet provided.

A suggested plate format is shown in Figure, below. The user may vary the location and type of wells present as necessary for each particular experiment.

1 2 3 4 5 6 7 8 9 10 11 12
A PC1 PC1 S1 S1 S9 S9 S17 S17 S25 S25 S33 S33
B PC2 PC2 S2
C PC3 PC3 S3
D PC4 PC4 S4
E PC5 PC5 S5
F PC6 PC6 S6
G 0 0 S7
H Blk Blk S8 S8 S16 S16 S24 S24 S32 S32 S40 S40
Blk - Blank Wells
0 - Zero Wells

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PC1-PC6 - Posive Control Wells
S1-S40 - Sample Wells

General Information
• Plate strips can be used in separate experiments if stored at 4°C properly in the resealable pouch.
• A minimum of two Blk, two zero wells, and two PC wells should be included in each assay.
• We recommend using Nuclear Extraction Kit for preparing your samples.

Performing the Assay
Binding of active NF-κB (p65) to the consensus sequence:
1. Equilibrate the plate and buffers to room temperature prior to opening. Remove the plate from the foil and select the number of strips needed. The 96-well plate supplied with this kit is ready to use.
2. Add the appropriate amount of reagents listed below to the designated wells as follows:
Blk - add 100 µl of CTFB to designated wells.
Zero Well - add 100 µl of CTFB to designated wells.
PC1-PC6 - Add 100 µl of PC dilutions to the appropriate wells.
S1-S40 - Add 90 µl of CTFB followed by 10 µl of sample to designated wells.
Competitor (optional) - Add 80 µl of CTFB prior to adding 10 µl of Transcription Factor NF-κB Competitor dsDNA to designated wells, followed by 10 µl of control cell lysate or sample.
3. Use the 96-Well Cover Sheet provided to seal the plate. Incubate overnight at 4°C without shaking or one hour at room temperature on an orbital shaker.
4. Empty the wells and wash five times with 200 µl of Wash Buffer (1X). After the final wash, tap the plate on a paper towel to remove any residual Wash Buffer. Addition of Transcription Factor NF-κB (p65) Primary Antibody
5. Dilute the Transcription Factor NF-κB (p65) Primary Antibody 1:100 in ABB (1X). Add 100 µl to each well except the Blk wells.
6. Seal the plate with the cover sheet.
7. Incubate the plate for one hour at room temperature on an orbital shaker.
8. Empty the wells and wash each well five times with 200 µl of Wash Buffer (1X). After the final wash,

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tap the plate three to five times on a paper towel to remove any residual wash buffer.
Addition of the Transcription Factor Goat Anti-Rabbit HRP Conjugate
9. Dilute the Transcription Factor Goat Anti-Rabbit HRP Conjugate 1:100 in ABB (1X). Add 100 µl to each well except the Blk wells.
10. Seal the plate with the cover sheet.
11. Incubate for one hour at room temperature on an orbital shaker.
12. Empty the wells and wash five times with 200 µl of Wash Buffer (1X). After the final wash, tap the plate three to five times on a paper towel to remove any residual wash buffer.
Develop and Read the Plate
13. Add 100 µl of Transcription Factor Developing Solution, to each well.
14. Seal the plate with the cover sheet, and incubate the plate for 30 minutes at room temperature on an orbital shaker protected from light.
15. Remove cover sheet and add 100 µl of Transcription Factor Stop Solution per well. The solution within the wells will change from blue to yellow.
16. Read absorbance at 450 nm within five minutes of adding the Transcription Factor Stop Solution.

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