ab289845 – NF-kappaB RelA/p65 Transcription Factor Activity Assay Kit (Colorimetric)

For the measurement of human NFkB p65 activation in nuclear extracts or cell lysates.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab289845

Storage and Stability

Store the kit at -20°C except the Positive Control, which should be stored at -80°C. Once the kit is opened, store the kit components as recommend in the Reagent Preparation section.

Materials Supplied

Item	Quantity	Storage Condition
Plate Coated with DNA Probes	1 unit	-20°C
Binding Buffer (5X)	2.2 mL	-20°C
DTT (100 mM)	100 µL	-20°C
Protease Inhibitor Cocktail	20 µL	-20°C
RelA/p65 Primary Antibody	200 µL	-20°C
Antibody Diluent Buffer	20 mL	-20°C
HRP Conjugate Stock	8 µL	-20°C
Wash Buffer (10X)	27 mL	-20°C
Competitor Oligo (20 pmole)	25 µL	-20°C
Non-Competitor Oligo (20 pmole)	25 µL	-20°C
TMB Substrate	10 mL	-20°C
Stop Solution	6 mL	-20°C
Positive Control	50 µL	-80°C
Plate Sealing Film	2 units	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- dH_2O
- Cell lysis buffer or Nuclear/Cytosol Fractionation Kit.
- Multi-well spectrophotometer (ELISA reader)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended
- Dounce Tissue Homogenizer
- Absorbent paper

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Plate Coated with DNA Probes: Do not open until ready to use. Bring to room temperature (RT) before use. After opening, immediately store the remaining unused strips at -20°C.

Binding Buffer (5X): Store at -20°C. Bring to RT before use. Prepare fresh Binding Buffer for the assay by adding 10 µL of 100 mM DTT and 2 µL of Proteinase Inhibitor Cocktail to 988 µL 5X Binding Buffer. Prepare enough reagents to add 100 µL/well. Use within 1 hr.

DTT (100 mM), Protease Inhibitor Cocktail, Competitor Oligo (20 pmol) and Non-Competitor Oligo (20 pmol): Divide into aliquots and store at -20 °C. Avoid repeated freeze-thaw cycles.

RelA/p65 Primary Antibody: Divide into aliquots and store at -20°C. Prepare RelA/p65 Primary Antibody working solution by adding 2 µL RelA/p65 Primary Antibody to 98 µL Antibody Diluent Buffer. Prepare enough reagents for the assay (100 µL/well). Keep on ice when in use.

HRP Conjugate Stock: Spin briefly before opening the vial. Prepare enough HRP Conjugate working solution to add 100 µL/well. For example, mix 4 µL of HRP Conjugate Stock with 7.5 mL Antibody Diluent Buffer for 70 assays. The HRP Conjugate working solution is stable at 4°C for 2 months.

Wash Buffer (10X): Bring to RT before use. Prepare 1X Wash Buffer for the assay. Prepare enough reagents for the assay. Diluted Wash Buffer can be stored for 1 month at 4°C.

TMB Substrate and Stop Solution: Ready to use. Store at 4°C.

Positive Control (2 µg/µL): Store at -80°C. Thaw on ice before use. Avoid repeated freeze-thaw cycles. Keep on ice when in use

Sample Preparation

Cell lysate: Homogenize pelleted cells (~5 x 10^5) with 100 µL ice-cold cell lysis buffer using Dounce Tissue Homogenizer and keep on ice for 10-15 min. Centrifuge samples at 12,000 x g and 4°C for 15 min and collect the supernatant.

Nuclear extract: Prepare nuclear extracts using a Nuclear/Cytosol Fractionation Kit or any preferred method.

Assay Protocol

1. **Transcription Factor Binding Reaction Mix Preparation**: Prepare four different Transcription Factor Binding Reaction Mixes as shown below.

Δ Note: *Mix enough reagents for the number of assays to be performed. The amount of Sample used per assay should be optimized by the researcher. A Positive Control should be included to confirm if the assay is working.*

	Sample or Positive Control	Specific Competitor	Non-Specific Competitor	Background Control
Bing Buffer (5X)	20 µL	20 µL	20 µL	20 µL
Sample or Positive Control	10 µL (20 µg)	10 µL (20 µg)	10 µL (20 µg)	---
Competitor Oligo (20 pmole)	---	1 µL	---	---
Non-Competitor Oligo (20 pmole)	---	---	1 µL	---
dH2O	70 µL	69 µL	69 µL	80 µL
Total Volume	100 µL	100 µL	100 µL	100 µL

2. Wash each well of the Plate Coated with DNA Probes, 3 times with 200 µL of 1X Wash buffer and discard the solution by decanting. Tap the inverted plate 3-5 times on a clean paper towel to remove any residual solution.
3. Add 100 µL of each Transcription Factor Binding Reaction Mix into appropriate wells. Cover the microtiter plate and incubate for 1 hr at RT with gentle orbital shaking (< 10 rpm).
4. Decant all the reagents and wash each well 3 times as described in step 2.
5. Add 100 µL of RelA/p65 Primary Antibody working solution to each well.

6. Cover the plate and mix well. Incubate the plate at RT for 1 hr with gentle orbital shaking (< 10 rpm).
7. Decant or aspirate all the reagents and wash each well 3 times as described in step 2.
8. Add 100 µL of HRP Conjugate working solution to each well.
9. Cover the plate and mix well. Incubate the plate at RT for 1 hr with gentle orbital shaking (< 10 rpm).
10. Decant or aspirate all the reagents and wash each well 3 times as described in step 2.
11. Decant the HRP Conjugate working solution and wash each well 3 times as described in step 2.
12. Add 100 µL of TMB Substrate to each well. Incubate up to 30 min without shaking, protected from light.
    Δ Note: *Optimal incubation time will vary for each experiment depending on amount of transcription factor present in the sample.*
13. Monitor the color development in the sample wells until it turns medium to dark blue.
    Δ Note: *Do not overdevelop.*
14. Add 50 µL Stop Solution to all wells and gently tap the plate to ensure thorough mixing.
    Δ Note: *The solution in the wells will change color from blue to yellow.*
15. Measure the absorbance at 450 nm within 5 min at RT.

Technical Support

For all technical or commercial enquiries please go to:

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