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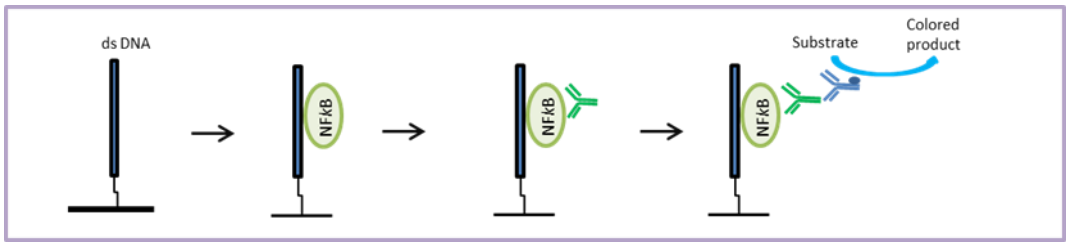
NFkB RelA/p65 Transcription Factor Activity Assay Kit (Colorimetric)

06/21

(Catalog # K2093-100; 100 assays; Store at Multiple Temperatures)

I. Introduction:

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) represents a family of inducible transcription factors, which regulates many biological processes including immune responses, cell growth, apoptosis etc. This family includes five structurally related members namely NF-κB1 (or p50), NF-κB2 (or p52), RelA (or p65), RelB and c-Rel, which are normally sequestered in the cytoplasm by a family of inhibitory proteins and are inactive. Upon stimulation, NF-κB proteins form homo- or heterodimers and are transported into the nucleus where they bind to specific DNA sequences of targeted genes and activate transcription. Traditionally, western blot is used to detect the expression of NFkB and electrophoretic mobility shift assay or reporter assays are used to measure the NFkB activity respectively. But some of these methods are time consuming, laborious, and require radioactivity. **BioVision's NFkB RelA/p65 Transcription Factor Activity Assay** is a 96-well plate based colorimetric assay to measure the activation of human NFkB p65 in nuclear extracts or cell lysates. The kit offers easy, rapid, sensitive and non-radioactive way to detect the activation of transcription factors in samples. In this assay, double stranded DNA sequence containing the NFkB p65 consensus binding site is coated on the 96-well plate. Active NFkB p65 in the cell lysate or the nuclear extract binds to the oligonucleotides on the plate. After the addition of RelA/p65 primary antibody that recognizes the NFkB p65-oligonucleotide complex, a HRP-conjugated secondary antibody is added followed by the addition of TMB substrate and a color signal is developed, which is measured at 450 nm.



II. Application:

- Semi-quantitative measurement of activation of human NFkB p65 in nuclear extracts or cell lysates.

III. Sample Types:

- Cell lysates
- Nuclear extracts

IV. Kit Contents:

Components	K2093-100	Cap Code	Part Number
Plate Coated with DNA Probes	1	--	K2093-100-1
Binding Buffer (5X)	2.2 ml	NM	K2093-100-2
DTT (100 mM)	100 µl	White	K2093-100-3
Protease Inhibitor Cocktail	20 µl	Amber	K2093-100-4
RelA/p65 Primary Antibody	200 µl	Green	K2093-100-5
Antibody Diluent Buffer	20 ml	WM	K2093-100-6
HRP Conjugate Stock	8 µl	Blue	K2093-100-7
Wash Buffer (10X)	27 ml	NM	K2093-100-8
Competitor Oligo (20 pmole)	25 µl	Orange	K2093-100-9
Non-Competitor Oligo (20 pmole)	25 µl	Red	K2093-100-10
TMB Substrate	10 ml	Amber/NM	K2093-100-11
Stop Solution	6 ml	Red	K2093-100-12
Positive Control	50 µl	Yellow	K2093-100-13
Plate Sealing Film	2	--	K2093-100-14

V. User Supplied Reagents and Equipment:

- dH_2O
- Cell lysis buffer or BioVision's Nuclear/Cytosol Fractionation Kit (BioVision Cat.No. K266).
- Multi-well spectrophotometer (ELISA reader)
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended
- Dounce Tissue Homogenizer (BioVision Cat.No.1998)
- Absorbent paper

VI. Storage Conditions and Reagent Preparation:

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 below. Briefly centrifuge all small vials prior to opening. Read the entire protocol before performing the assay

- **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.

VII. RelA/p65 Transcription Factor Activity Assay Protocol:

1. Sample Preparation: ***Cell lysate preparation:*** Homogenize pelleted cells (~5 x 10^5) with 100 µl ice-cold cell lysis buffer using Dounce Tissue Homogenizer (BioVision Cat.No. 1998) 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 preparation:*** Prepare nuclear extracts using BioVision's Nuclear/Cytosol Fractionation Kit (BioVision Cat. No. K266) or any preferred method.

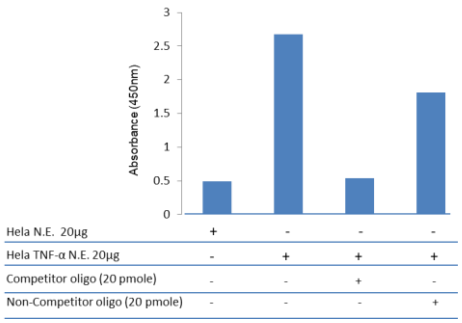
2. Transcription Factor Binding Reaction Mix Preparation: Prepare four different Transcription Factor Binding Reaction Mixes as shown below. **Notes:** Mix enough reagents for the number of assays to be performed. The amount of Sample used per assay should to be optimized by the researcher. A Positive Control should be included to confirm if the assay is working.

	Sample or Positive Control	Specific Competitor	Non-Specific Competitor	Background Control
Binding 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	--
dH_2O	70 µl	69 µl	69 µl	80 µl
Total Volume	**100 µl**	**100 µl**	**100 µl**	**100 µl**

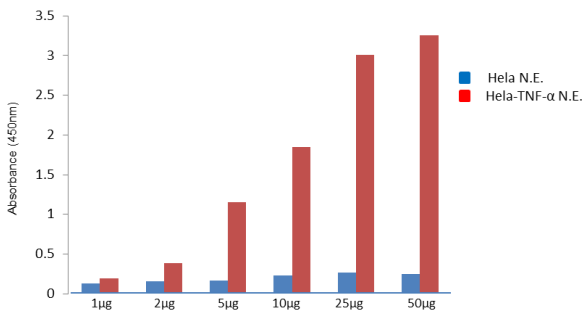
3. Wash each well of the **Plate Coated with DNA Probes,** 3 times with 200 µl of 1X Wash buffer and discard the solution by decanting. Tap the inverted plate 3-5 times on a clean paper towel to remove any residual solution.
4. Add 100 µl of each **Transcription Factor Binding Reaction Mix** into appropriate wells. Cover the microtiter plate and incubate for 1 hr at RT with gentle orbital shaking (< 10 rpm).
5. Decant all the reagents and wash each well 3 times as described in step 3.
6. Add 100 µl of **RelA/p65 Primary Antibody working solution** to each well
7. Cover the plate and mix well. Incubate the plate at RT for 1 hr with gentle orbital shaking (< 10 rpm).
8. Decant or aspirate all the reagents and wash each well 3 times as described in step 3.
9. Add 100 µl of **HRP Conjugate working solution** to each well.
10. Cover the plate and mix well. Incubate the plate at RT for 1 hr with gentle orbital shaking (< 10 rpm).
11. Decant or aspirate all the reagents and wash each well 3 times as described in step 3.
12. Decant the HRP Conjugate working solution and wash each well 3 times as described in step 3.
13. Add 100 µl of **TMB Substrate** to each well. Incubate up to 30 min without shaking, protected from light. **Note:** Optimal incubation time will vary for each experiment depending on amount of transcription factor present in the sample.
14. Monitor the color development in the sample wells until it turns **medium to dark blue**. **Note:** Do not overdevelop.
15. Add 50 µl **Stop Solution** to all wells and gently tap the plate to ensure thorough mixing. **Note:** The solution in the wells will change Color from blue to yellow.
16. Measure the absorbance at 450 nm within 5 min at RT.

VIII. Typical Data:

(A)



(B)



Figures: Transcription factor activity assay using nuclear extracts of **A & B.** Hela cells were treated with TNF-α for 30 min. Assay was performed following the assay kit protocol.

IX. Related Products:

HDAC-5 Inhibitor Screening Kit (Fluorometric) (K171)
p53 Nuclear Translocation Assay Kit (Cell-Based) (K961)
pCAF Inhibitor Screening Kit (Fluorometric) (K345)
TFEB Transcription Factor Activity Assay Kit (Colorimetric) (K2088)

FOR RESEARCH USE ONLY! Not to be used on humans.