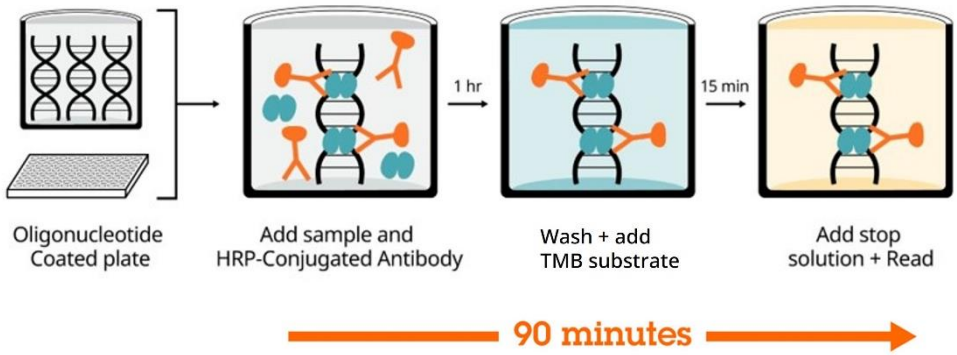
ab324202 – NFkB p50 Transcription Factor Activity Assay

For the semiquantitative measurement of activated NFkB p50 in Human Nuclear extract, Cell lysate, and Tissue lysate.
For research use only - not intended for diagnostic use.



For overview, typical data and additional information please visit:
http://www.abcam.com/ab324202

Storage and Stability: Store kit at -20°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Reagent preparation section.

Materials Supplied

Item	Quantity	Storage Condition (Upon receipt)	Storage Condition (After Prep)
NFkB Capture DNA Probe Coated Plate	96 wells	-20°C	-20°C
NFkB Competitor DNA probe	25 µL	-20°C	-20°C
NFkB Non-Competitor DNA probe	25 µL	-20°C	-20°C
NFkB p50 Detector Antibody	200 µL	-20°C	-20°C
NFkB Positive Control	1vial Lyophilized	-20°C	-80°C
5x Binding Buffer	2.2 mL	-20°C	-20°C
100x DTT	100 µL	-20°C	-20°C
200x Protease Inhibitor Cocktail	150 µL	-20°C	-20°C
Antibody Diluent Buffer	20 mL	-20°C	+4°C
Wash Buffer PT 10X	20 mL	+4°C	+4°C
TMB Development Solution	12 mL	+4°C	+4°C
Stop Solution	12 mL	+4°C	+4°C
Microplate Seal Film	1		

Materials Required, Not Supplied

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

Microplate reader capable of measuring absorbance at 450 or 600 nm.
Nuclear extraction kit (ab219177) or Extraction Buffer 5X PTR (ab193970)
Method for determining protein concentration (BCA assay recommended).
Deionized water.
Multi- and single-channel pipettes.
Tubes for aliquoting.
Plate shaker for all incubation steps.

Reagent Preparation

- Equilibrate NFKB Capture DNA probe coated plate, 5x Binding buffer, 10x wash buffer, TMB solution and Stop solution to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment. Detector Antibody have only been tested for stability in the provided formulation.

Capture DNA Probe coated plate: Do not open until ready to use. Bring to room temperature before use. After opening immediately store remaining unused strips at -20°C.

100x DTT, 200x Protease Inhibitor cocktail, Competitor DNA Probe and Non-Competitor DNA Probe: Divide into aliquots and store at -20°C. Avoid freeze-thaw cycles.
Note: *Competitor DNA Probe contains the wildtype consensus sequence and will compete with the Capture DNA Probe immobilized on the plate for NFkB binding. Non-Competitor DNA Probe contains a mutated consensus sequence and should not compete with Capture DNA Probe. Competitor and Non-competitor DNA Probes are provided for a competition experiment to check the specificity of the assay. The DNA probe competition is used as a control and should be run with each new sample type.*

Positive Control: Reconstitute positive control by adding 110 µL of deionized water. Mix thoroughly and gently. Keep on ice while in use. Aliquot and store at -80°C. Thaw aliquots on ice.
Note: *Reconstituted positive control storage at –20°C will result in signal loss.*

Detector Antibody: Prepare Detector Antibody working solution by adding 2 µL of Detector antibody to 48 µL of Antibody Diluent Buffer. Prepare enough reagent for 50 µL per well.

Binding Buffer: Store at -20°C. Bring to room temperature before use. Buffer may have small precipitants; this will not affect performance. Prepare fresh Binding buffer for the assay by adding 10 µL of 100x DTT and 2 µl of 200x Protease inhibitor cocktail to 988 µL of 5x Binding Buffer. Prepare enough reagent for 20 µL per well. Make fresh and use within 12 hours.

1X Wash Buffer PT: Prepare 1X Wash Buffer PT by diluting Wash Buffer PT 10X with deionized water. To make 50 mL 1X Wash Buffer PT combine 5 mL Wash Buffer PT 10X with 45 mL deionized water. Mix thoroughly and gently.

Sample Preparation

Nuclear Extract
Prepare nuclear extracts using Abcam's Nuclear extraction kit (ab219177). The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in Nuclear Extraction Buffer.

Preparation of extracts from cell pellets: Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. Typical centrifugation conditions for cells are 500 x g for 5 minutes at 4°C. Rinse cells twice with PBS. Solubilize pellet at $2x10^7$ cell/mL in chilled 1X Cell Extraction Buffer PTR. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Preparation of extracts from adherent cells by direct lysis (alternative protocol): Remove growth media and rinse adherent cells 2 times in PBS. Solubilize the cells by addition of chilled 1X Cell Extraction Buffer PTR directly to the plate (use 750 µL - 1.5 mL 1X Cell Extraction Buffer PTR per confluent 15 cm diameter plate). Scrape the cells into a microfuge tube and incubate the lysate on ice for 15 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.
Preparation of extracts from tissue homogenates: Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (Dounce homogenizer recommended). Homogenize 100 to 200 mg of wet tissue in 500 µL – 1 mL of chilled 1X Cell Extraction Buffer PTR. For lower amounts of tissue adjust volumes accordingly. Incubate on ice for 20 minutes. Centrifuge at 18,000 x g for 20 minutes at 4°C. Transfer the supernatants into clean tubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C. The sample protein concentration in the extract may be quantified using a protein assay. Dilute samples to desired concentration in 1X Cell Extraction Buffer PTR.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. Rinse the plate 3x with 1x Wash buffer PT prior to adding reagents.
Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at -20°C.
For each assay performed, a minimum of two wells must be used as the positive control.
For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.
We recommend that you assay all controls and samples in duplicate.

1. Prepare four different transcription factor binding reaction mixes as shown below.
   Note: *Mix enough reagents for the number of assays to be performed in duplicates. The amount of Sample used per assay should be optimized by the researcher. For Nuclear Extract recommended starting concentration is 3 µg/well. For Cell lysates and Tissue lysates the amount of protein required can be significantly more than nuclear extracts.*

	Sample	Specific Competitor	Non-Specific Competitor	Positive Control	Background Control
Binding Buffer	20 µL	20 µL	20 µL	20 µL	20 µL
Sample or Positive Control	2 -29 µL	2 -29 µL	2 -29 µL	3 µL	–
Competitor DNA Probe	–	1 µL	–	–	–
Non-competitor DNA Probe	–	–	1 µL	–	–
Detector Antibody Working Solution	50 µL	50 µL	50 µL	50 µL	50 µL
dH20	To 100 µL	To 100 µL	To 100 µL	27 µL	30 µL
Total volume	100 µL	100 µL	100 µL	100 µL	100 µL

2. Wash each well of the Capture DNA probe coated plate 3X with 200 µL of 1X Wash Buffer PT and discard the solution by aspirating or decanting. Tap the inverted plate 3-5 times on a clean paper towel to remove any residual solution. Complete removal of liquid at each step is essential for good performance.
3. Add 100 µL of each transcription factor binding reaction mix into appropriate wells. Cover the microplate and incubate for 1hour at room temperature on a plate shaker set to 400 rpm.
4. Decant all reagents and wash each well 3 times as described in step 2.
5. Add 100 µL of TMB Development Solution to each well and incubate for 15 minutes in the dark on a plate shaker set to 400 rpm.
   Given variability in laboratory environmental conditions, optimal incubation time may vary between 5 and 20 minutes.
   Note: *The addition of Stop Solution will change the color from blue to yellow and enhance the signal intensity about 3X. To avoid signal saturation, proceed to the next step before the high concentration of the standard reaches a blue color of O.D.600 equal to 1.0.*
6. Add 100 µL of Stop Solution to each well. Shake plate on a plate shaker for 1 minute to mix. Record the OD at 450 nm. This is an endpoint reading.

7. Alternative to 5 – 6: Instead of the endpoint reading at 450 nm, record the development of TMB Substrate kinetically. Immediately after addition of TMB Development Solution begin recording the blue color development with elapsed time in the microplate reader prepared with the following settings:

Mode	Kinetic
Wavelength:	600 nm
Time:	Up to 20 min
Interval:	20 sec- 1 min
Shaking:	Shake between readings

Note *that an endpoint reading can also be recorded at the completion of the kinetic read by adding 100 µL Stop Solution to each well and recording the OD at 450 nm.*

Additional information

TYPICAL SAMPLE VALUES

Sensitivity:

The MDD (minimal detectable dose) is the lowest concentration of TNF alpha treated Hela Nuclear Extract to produce signal above background.
The MDD is 0.1875 µg/ well.

Precision

Mean coefficient of variations of values of NFkB p50 from 3 concentrations of TNF alpha treated Hela Nuclear extract.

	Intra-assay	Inter-assay
N=	**8**	**3**
CV (%)	**2**	**9**

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:
www.abcam.com/protocols/the-complete-elisa-guide

Technical Support

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