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## Datasheet for ABIN1380147

# NF-kB p65 ELISA Kit



#### Overview

Quantity:	2 x 96 tests
Target:	NF-kB p65 (NFkBP65)
Binding Specificity:	pSer529
Reactivity:	Human, Rat, Mouse
Method Type:	Cell ELISA
Application:	ELISA
Product Details	
Sample Type:	Cell Culture Cells
Analytical Method:	Qualitative
Detection Method:	Colorimetric
Specificity:	The Anti-NF-kappaB p65 (Phospho-Ser529) antibody is a rabbit polyclonal antibody. It was
	tested on Western Blots for specificity. A single protein band was detected. This protein band
	can be blocked by the synthesized immunogen peptide.
	The Anti-NF-kappaB p65 (Phospho-Ser529) antibody is highly specific for the phospho-peptide
	in comparison to the non-phospho peptide counterpart, through an ELISA.
	The Anti-NF-kappaB p65 antibody is a rabbit polyclonal antibody. It was tested on Western
	Blots for specificity. A single protein band was detected. This protein band can be blocked by
	the synthesized immunogen peptide.

detected from all three lysates.

The Anti-GAPDH antibody is a mouse monoclonal antibody. It was tested on Western Blots with

the tissue lysates from human, mouse, and rat for specificity. A single protein band was

Characteristics:

1) Cell Line: The cell line must express the target protein. This protocol can be used directly for adherent cells. For suspension cells and loosely attached cells, two steps are required: Coat the plates with 100  $\mu$ L of 10  $\mu$ g/mL Poly-L-Lysine to each well of the 96-well plate for 30 minutes at 37°C before proceeding to Step 1 of Assay Protocol. Use 8% formaldehyde to fix the cells on Step 5 of Assay Protocol.

2) Cell Number and Sensitivity: The number of cells plated onto the 96-well plates depends on the expression level of NF-kappaB p65 protein in the cells, cell size, treatment conditions and incubation time. The cells used for testing should be around 75-90% confluent. Typically for HeLa cells, seed 30,000 cells per well overnight for treatment the following day. The NF-kappaB p65 (Phospho-Ser529) Colorimetric Cell- Based ELISA Kit can detect Phospho-NF-kappaB p65 expression in as little as 5,000 HeLa cells.

3) Cell Treatment: The cells can be treated with inhibitors, activators, stimulators (ie. chemicals, proteins/peptides) or a combination of the substances listed above. The cells can be treated with UV and serum starvation to meet the needs of the end-user.

4) Positive and Negative Controls: Mouse Anti-GAPDH antibody (included) should be used to detect the internal positive controls for normalization of OD values of the target protein. The negative controls are HRP-Conjugated Anti-Rabbit IgG antibody and HRP- Conjugated Anti-Mouse IgG antibody alone in different wells (without the primary antibodies). Both positive and negative controls should be performed in the same plate with the Phospho-NF-kappaB p65 target experiments.

5) Accuracy and Precision: Each condition should be performed in duplicate or in triplicate.

Components:

96-Well Cell Culture Clear-Bottom Microplate: 2 Plates

TBS: 24 mL (10x), Clear

Quenching Buffer: 24 mL (1x), Clear Blocking Buffer: 50 mL (1x), Clear Wash Buffer: 50 mL (10x), Clear

Anti-NF-kappaB p65 (Phospho-Ser529) antibody (Rabbit Polyclonal): 60 µl (100x), Red

Anti-NF-kappaB p65 antibody (Rabbit Polyclonal): 60 μl (100x), Purple

Anti-GAPDH antibody (Mouse Monoclonal): 60 µl (100x), Green HRP-Conjugated Anti-Rabbit IgG antibody 12 mL (1x), Glass HRP-Conjugated Anti-Mouse IgG antibody 12 mL (1x), Glass

## **Product Details**

	Primary antibody Diluent: 12 mL (1x), Clear
	Ready-to-Use Substrate: 12 mL (1x), Brown
	Stop Solution: 12 mL (1x), Clear
	Crystal Violet Solution: 12 mL (1x), Glass
	SDS Solution: 24 mL (1x), Clear
	Adhesive Plate Seals: 4 Seals
Material not included:	The following materials and equipment are NOT provided in this kit but are necessary to
	successfully conduct the experiment:
	Microplate reader able to measure absorbance at 450 nm and/or 595 nm for Crystal Violet Cell
	Staining (Optional)
	Micropipettes with capability of measuring volumes ranging from 1 $\mu L$ to 1 mL
	37% formaldehyde

# Target Details

Target:	NF-kB p65 (NFkBP65)
Alternative Name:	NF-kappaB p65 (NFkBP65 Products)
Molecular Weight:	60219
OMIM:	164014
UniProt:	Q04206
Pathways:	NF-kappaB Signaling, RTK Signaling, TCR Signaling, TLR Signaling, Fc-epsilon Receptor Signaling Pathway, Neurotrophin Signaling Pathway, Activation of Innate immune Response, Cellular Response to Molecule of Bacterial Origin, Hepatitis C, Toll-Like Receptors Cascades, S100 Proteins

# **Application Details**

Comment:	Phospho-NF-кВ p65: Human:pSer-529, Mouse:pSer-527, Rat:pSer-528
Plate:	Uncoated
Protocol:	The Colorimetric Cell-Based ELISA Kit allows for the detection of various target proteins and the
	effects that certain stimulation conditions have on target protein expression in different cell
	lines. Qualitative determination of target protein concentration is achieved by an indirect ELISA
	format. In essence, the target protein is captured by target-specific primary (1st) antibodies
	while the HRP-conjugated secondary (2nd) antibodies bind the Fc region of the 1st antibody.

Through this binding, the HRP enzyme conjugated to the 2nd antibody can catalyze a colorimetric reaction upon substrate addition. Due to the qualitative nature of the Cell-Based ELISA, multiple normalization methods are described:

- 1) a monoclonal antibody specific for human GAPDH is included to serve as an internal positive control in normalizing the target absorbance values.
- 2) Following the colorimetric measurement of HRP activity via substrate addition, the Crystal Violet whole-cell staining method is used to determine cell density. After staining, the results can be analyzed by normalizing the absorbance values to cell amounts, by which the plating difference can be adjusted.
- 3) If a phosphorylated target is being detected, an antibody against the non- phosphorylated counterpart will be provided for normalization purposes. The absorbance values obtained for the non-phosphorylated target can be used to normalize the absorbance values for the phosphorylated target.

NF-kappaB p65 (Phospho-Ser529) Colorimetric Cell-Based ELISA

The NF-kappaB p65 (Phospho-Ser529) Cell-Based ELISA Kit is a convenient, lysate-free, high throughput and sensitive assay kit that can monitor NF-kappaB p65 protein phosphorylation and expression profile in cells. The kit can be used for measuring the relative amounts of phosphorylated NF-kappaB p65 in cultured cells as well as screening for the effects that various treatments, inhibitors (ie. siRNA or chemicals), or activators have on NF-kappaB p65 phosphorylation.

### Reagent Preparation:

Note: Please remember to allow all solutions to warm up to room temperature prior to use.

1x TBS: 1x TBS is used to wash cells seeded on the plate. 1x TBS can be prepared by adding 1 volume of 10x TBS provided in the kit to 9 volumes of ddH20.

Fixing Solution: This solution is NOT provided. Fixing Solution is used to fix cells after cell culture. It is prepared by adding formaldehyde to 1x TBS with light mixing. The 4% formaldehyde is used for adherent cells and 8% formaldehyde is used for suspension cells and loosely attached cells.

Quenching Buffer: This solution is provided as ready-to-use. Quenching Buffer is used to inactivate the endogenous peroxidase activity of the seeded cells.

Blocking Buffer: This solution is provided as ready-to-use. Blocking Buffer is used to block additional binding sites in each well.

Wash Buffer: This buffer is provided as a 10x solution. 1x Wash Buffer can be prepared by adding 1 volume of 10x Wash Buffer provided in the kit to 9 volumes of ddH2O.

100x Anti-NF-kappaB p65 (Phospho-Ser529) antibody: This antibody is a rabbit polyclonal antibody. This antibody was tested to be specific for the NF-kappaB p65 protein

phosphorylated at Ser529. The supplied antibody is a 100x solution. Make 1:100 dilutions in Primary antibody Diluent prior to use. The diluted primary antibody can be stored at 4°C for up to two weeks.

100x Anti-NF-kappaB p65 antibody: This antibody is a rabbit polyclonal antibody. This antibody was tested to be specific for the NF-kappaB p65 protein. The supplied antibody is a 100x solution. Make 1:100 dilutions in Primary antibody Diluent prior to use. The diluted primary antibody can be stored at 4°C for up to two weeks.

100x Anti-GAPDH antibody: This antibody is a mouse monoclonal antibody. This antibody was tested to be specific for GAPDH. The supplied antibody is a 100x solution. Make 1:100 dilutions in Primary antibody Diluent prior to use. The diluted primary antibody can be stored at 4°C for up to two weeks.

HRP-Conjugated Anti-Rabbit IgG antibody: This solution is provided as ready-to-use. HRP-Conjugated Anti-Rabbit IgG antibody is used as the secondary antibody to detect the target-bound, primary rabbit antibodies.

HRP-Conjugated Anti-Mouse IgG antibody: This solution is provided as ready-to-use. HRP-Conjugated Anti-Mouse IgG antibody is used as the secondary antibody to detect the target-bound, primary mouse antibodies.

Primary antibody Diluent: This solution is provided as ready-to-use. Use this solution to dilute the provided antibodies.

Ready-to-Use Substrate: This solution is provided as ready-to-use. Ready-to-Use Substrate must be warmed to room temperature before use. Keep away from light as this solution is light-sensitive.

Stop Solution: This solution is provided as ready-to-use. Stop Solution must be handled with caution as it contains 2 N Sulfuric Acid (H2SO4) and is corrosive. Wear eye protection and gloves when handling.

Crystal Violet Solution: This solution is provided as ready-to-use. Crystal Violet is an intense stain used to stain cell nuclei. Avoid contact with skin and clothing.

SDS Solution: This solution is provided as ready-to-use. SDS is used to solubilize the Crystal Violet in preparation for cell staining. Store this solution at room temperature or warm up to room temperature if stored at 4°C.

#### Assay Procedure:

Note: Please read the whole manual before performing the experiment.

1) Seed 200  $\mu$ L of 20,000 adherent cells in culture medium in each well of a 96-well plate. The plates included in the kit are sterile and treated for cell culture. For suspension cells and loosely attached cells, coat the plates with 100  $\mu$ L of 10  $\mu$ g/mL Poly-L-Lysine (not included) to each well of a 96-well plate for 30 minutes at 37°C prior to adding cells.

- 2) Incubate the cells for overnight at 37°C, 5% CO2.
- 3) Treat the cells as desired.
- 4) Remove the cell culture medium and rinse with 200  $\mu L$  of 1x TBS, twice.
- 5) Fix the cells by incubating with 100  $\mu$ L of Fixing Solution for 20 minutes at room temperature. The 4% formaldehyde is used for adherent cells and 8% formaldehyde is used for suspension cells and loosely attached cells. During the incubation, the plates should be sealed with Parafilm. Note: Fixing Solution is volatile. Wear appropriate personal protection equipment (mask, gloves and glasses) when using this chemical.
- 6) Remove the Fixing Solution and wash the plate 3 times with 200  $\mu$ L 1x Wash Buffer for five minutes each time with gentle shaking on the orbital shaker. The plate can be stored at 4°C for a week. Note: For all wash steps, tap the plate gently on absorbent papers to remove the solution completely.
- 7) Add 100 µL Quenching Buffer and incubate for 20 minutes at room temperature.
- 8) Wash the plate 3 times with 1x Wash Buffer for 5 minutes at a time, with gentle shaking on the shaker.
- 9) Add 200  $\mu$ L of Blocking Buffer and incubate for 1 hour at room temperature. 10) Wash 3 times with 200  $\mu$ L of 1x Wash Buffer for 5 minutes at a time, with gentle shaking on the shaker.
- 11) Add 50  $\mu$ L of 1x primary antibodies (Anti-NF-kappaB p65 (Phospho- Ser529) antibody, Anti-NF-kappaB p65 antibody and/or Anti-GAPDH antibody) to the corresponding wells, cover with Parafilm and incubate for 16 hours (overnight) at 4°C. If the target expression is known to be high, incubate for 2 hours at room temperature with gentle shaking on the shaker.
- 12) Wash 3 times with 200  $\mu$ L of 1x Wash Buffer for 5 minutes at a time, with gentle shaking on the shaker.
- 13) Add 50 µL of 1x secondary antibodies (HRP-Conjugated Anti- Rabbit IgG antibody and/or HRP-Conjugated Anti-Mouse IgG antibody) to corresponding wells and incubate for 1.5 hours at room temperature with gentle shaking on the shaker. Note: Add HRP-Conjugated Anti-Rabbit IgG antibody to the wells incubated with Anti-NF-kappaB p65 (Phospho-Ser529) antibody (rabbit, polyclonal) and/or Anti-NF-kappaB p65 (rabbit, polyclonal) and add HRP-Conjugated Anti-Mouse IgG antibody to the wells incubated with Anti-GAPDH antibody (mouse, monoclonal).
- 14) Wash 3 times with 200  $\mu$ L of 1x Wash Buffer for 5 minutes at a time, with gentle shaking on the shaker.
- 15) Add 50  $\mu$ L of Ready-to-Use Substrate to each well and incubate for 30 minutes at room temperature in the dark with gentle shaking on the shaker. Note: Ready-to-Use Substrate is a light-sensitive reagent. Keep away from light.
- 16) Add 50 µL of Stop Solution to each well and read OD at 450 nm immediately using the

microplate reader. Optional: Crystal Violet Cell Staining Crystal Violet binds to cell nuclei and gives absorbance readings proportional to cell counts at 595 nm.

- 17) After finishing reading the absorbance at 450 nm, wash the plate twice with 200  $\mu$ L of Wash Buffer and twice with 200  $\mu$ L of 1x TBS for 5 minutes each. Tap the plates on paper towel to remove the excess liquid. Let plate air dry for 5 minutes at room temperature.
- 18) Add 50  $\mu$ L of Crystal Violet Solution to each well, incubate for 30 minutes at room temperature on the shaker. Note: Crystal Violet is an intense stain. Avoid contact with skin and clothing.
- 19) Flick the plate to remove Crystal Violet Solution, rinse the plate by filling the wells with running tap water, and wash the plate with 200  $\mu$ L of 1x TBS 3 times, 5 minutes each with gently shaking on the shaker.
- 20) Add 100 µL of SDS Solution into each well and incubate on the shaker at room temperature for 1 hour.
- 21) Read absorbance at 595 nm with microplate reader. If absorbance is too high, the solubilized Crystal Violet Solution can be diluted 10 times with H2O on a separate 96-well plate.

#### Calculation of Results:

Anti-NF-kappaB p65 antibody normalization: The OD values obtained for the phosphorylated target protein can be normalized using the OD values obtained for the non-phosphorylated target protein via the proportion, OD450 (Anti-NF-kappaB p65 P-Ser529 antibody)/OD450 (Anti-NF-kappaB p65 antibody).

GAPDH normalization: The OD450 values obtained for the target protein (phosphorylated and non-phosphorylated) can be normalized using the OD450 values obtained for GAPDH.

Crystal Violet Staining normalization: The measured OD450 readings can be normalized using the OD595 values via the proportion, OD450/OD595.

#### Assay Precision:

Cell-Based

This ELISA kit is intended for research purposes only, NOT for diagnostic or clinical procedures of any kind.

Materials included in this kit should NOT be used past the expiration date on the kit label.

Reagents or substrates included in this kit should NOT be mixed or substituted with reagents or substrates from any other kits.

Variations in pipetting technique, washing technique, operator laboratory technique, kit age, incubation time or temperature may cause differences in binding affinity of the materials provided.

The assay is designed to eliminate interference and background by other cellular macromolecules or factors present within any biological samples. However, the possibility of

# **Application Details**

	background noise cannot be fully excluded until all factors have been tested using the assay kit
Restrictions:	For Research Use only
Handling	
Precaution of Use:	Reagents provided in this kit may be harmful if ingested, inhaled or absorbed through the skin.
	Fixing Solution contains formaldehyde. Formaldehyde is known to be a highly toxic reagent.
	Personal protection is strongly recommended while working with this chemical.
	Stop Solution contains 2 N Sulfuric Acid (H2SO4) and is an extremely corrosive agent. Please
	wear proper eye, hand and face protection when handling this material. When the experiment is
	finished, be sure to rinse the plate with copious amounts of running water to dilute the Stop
	Solution prior to disposing the plate or strips.
	Crystal Violet is an intense stain reagent. Avoid contact with clothing.
Storage:	4 °C
Storage Comment:	Upon receipt, the kit should be stored at 4°C. The un-opened kit will be stable for up to 6
	months from the date of shipment if stored at 4°C. Diluted Anti-NF-kappaB p65 (Phospho-
	Ser529) antibody, Anti-NF-kappaB p65 antibody and diluted Anti-GAPDH antibody can each be
	stored at 4°C for up to two weeks. HRP-Conjugated Anti-Rabbit IgG antibody and HRP-
	Conjugated Anti-Mouse IgG antibody will be stable at 4°C for up to six months. The SDS
	Solution should be stored at room temperature or warmed up to room temperature if stored at
	4°C.
Expiry Date:	6 months