Datasheet for ABIN2748433

NF-kB p65 ELISA Kit

Go to Product page

1 Image

Overview

Quantity:	2 x 96 tests
Target:	NF-kB p65 (NFkBP65)
Binding Specificity:	pSer536, total
Reactivity:	Human, Rat, Mouse
Method Type:	Sandwich ELISA
Application:	ELISA

Product Details

Purpose:	Human/Mouse/Rat Phospho-NF-KB p65 (S536) ELISA and Total NF-KB p65 Kit. This assay semi-quantitatively measures phophorylated NF-KB p65 (Ser536) and Total NF-KB p65 in lysate samples.
Sample Type:	Cell Lysate, Tissue Lysate
Analytical Method:	Semi-Quantitative
Detection Method:	Colorimetric
Specificity:	The antibody pair provided in this kit recognizes human NF-KB p65 phosphorylated at site Serine-536 and Total NF-KB p65.
Characteristics:	• Simultaneously measure Phosphorylated protein and pan protein in one experiment (for normalization purpose) • Screen numerous different cell lysates without performing a Western Blot analysis • Minimal hands-on time, convenient, and non-radioactive material
Components:	• Pre-Coated 96-well Strip Microplate

Product Details

- Wash Buffer
- Anti-Phospho Antibody
- Anti-Pan Antibody
- HRP-Conjugated Secondary Antibody
- Streptavidin-Conjugated HRP
- Assay Diluent
- TMB One-Step Substrate
- Stop Solution
- Lysis Buffer
- Positive Control Sample

Material not included:

- Distilled or deionized water
- 100 mL and 1 liter graduated cylinders
- Tubes to prepare sample dilutions
- Protease and Phosphatase inhibitors
- Precision pipettes to deliver 2 µL to 1 mL volumes
- Adjustable 1-25 mL pipettes for reagent preparation
- Benchtop rocker or shaker
- Microplate reader capable of measuring absorbance at 450 nm

Target Details

Target:	NF-kB p65 (NFkBP65)
Alternative Name:	NFKBP65 (NFkBP65 Products)
Background:	Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-KB p65) phosphorylated at Serine-536 and total NF-KB p65.
Gene ID:	5970
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

Plate:	Pre-coated
Protocol:	1. Prepare all reagents and samples as instructed in the manual. 2. Add 100 µL of sample or positive control to each well.

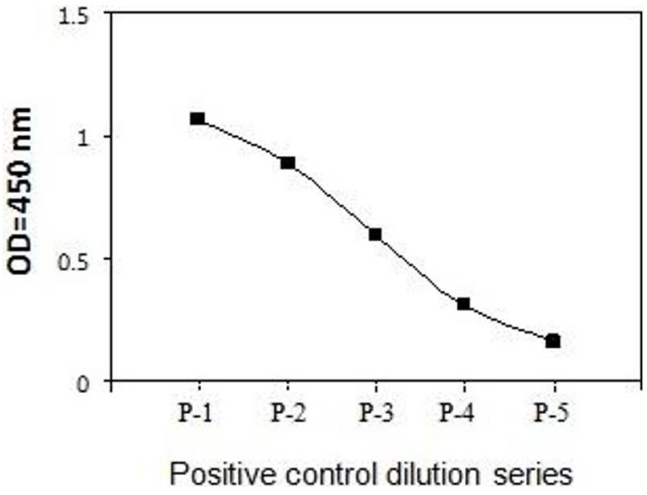
Application Details

3. Incubate 2.5 h at RT or O/N at 4 °C.
4. Add 100 µL of prepared primary antibody to each well.
5. Incubate 1 h at RT.
6. Add 100 µL of prepared 1X HRP-Streptavidin to each well.
7. Incubate 1 h at RT.
8. Add 100 µL of TMB One-Step Substrate Reagent to each well.
9. Incubate 30 min at RT.
10. Add 50 µL of Stop Solution to each well.
11. Read at 450 nm immediately.

Assay Procedure:	Prepare all reagents and samples as instructed in the manual. Add 100 µL of sample or positive control to each well. Incubate 2.5 h at RT or O/N at 4 °C. Add 100 µL of prepared primary antibody to each well. Incubate 1 h at RT. Add 100 µL of prepared 1X HRP-Streptavidin to each well. Incubate 1 h at RT. Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate 30 min at RT. Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.
Restrictions:	For Research Use only

Handling

Storage:	-20 °C
Storage Comment:	Upon receipt, the kit should be stored at -20 °C. Please use within 6 months from the date of shipment. After initial use, Wash Buffer Concentrate (Item B), Assay Diluent (Item E), TMB One-Step Substrate Reagent (Item H), HRP-Streptavidin (Item G), Stop Solution (Item I) and Cell Lysate Buffer (Item J) should be stored at 4 °C to avoid repeated freeze-thaw cycles. Return unused wells to the pouch containing desiccant pack, reseal along entire edge and store at -20 °C. Reconstituted Positive Control (Item K) should be stored at -70 °C.
Expiry Date:	6 months



ELISA

Image 1. HeLa cells were treated with TNFa and Calyculin A at 37°C for 5 min. Cells were solubilzed at 4 x 107 cells/ml in Cell Lysate Buffer. Serial dilutions of lysates were analyzed in this ELISA.

