

Datasheet for ABIN4889784

NF- κ B p65 ELISA Kit**3** Images[Go to Product page](#)

Overview

Quantity:	96 tests
Target:	NF- κ B p65 (NF κ BP65)
Reactivity:	Human
Method Type:	DNA-Binding ELISA
Application:	ELISA

Product Details

Purpose:	Human NF- κ B p65 Transcription Factor Activity Assay. This assay uses a dsDNA coated plate with canonical NF- κ B binding sequences to semi-quantitatively detect active NF- κ B in lysates or nuclear extracts. Only available in North America.
Sample Type:	Cell Lysate, Nuclear Extract
Analytical Method:	Semi-Quantitative
Detection Method:	Colorimetric
Specificity:	The oligonucleotide/antibody pair provided in this kit recognizes human NF- κ B p65 in whole lysates and nuclear extracts.
Characteristics:	<ul style="list-style-type: none">• Specific transcription factor-DNA binding assay• Perfect alternative to EMSA• Easy to perform in an ELISA format• Non-radioactive assay• High throughput (96 well plate format)• Assay can be completed within 5 hours

Product Details

Components:	<ul style="list-style-type: none">• 96-well Strip Microplate pre-coated with DNA probes• DNA Binding Buffer• Positive Control Sample• Specific Competitor DNA probe• Non-specific Competitor DNA probe• Assay Reagent• DTT• Wash Buffer• Primary Antibody• HRP-conjugated Secondary Antibody• TMB One-Step Substrate Reagent• Stop Solution
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Material not included:	<ul style="list-style-type: none">• Distilled or deionized water• 100 mL and 1 liter graduated cylinders• Tubes to prepare sample dilutions Absorbent paper• 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
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Target Details

Target:	NF-kB p65 (NFkBP65)
Alternative Name:	NFKBP65 (NFkBP65 Products)
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

Application Notes:	Optimal working dilution should be determined by the investigator.
Sample Volume:	100 µL
Plate:	Pre-coated

Application Details

Protocol:	<ol style="list-style-type: none">1. Prepare all reagents and samples as instructed in the manual.2. Add 100 μL of sample or positive control to each well.3. Incubate 2 h at RT or O/N at 4 $^{\circ}$C.4. Add 100 μL of prepared primary antibody to each well.5. Incubate 1 h at RT.6. Add 100 μL of prepared HRP-secondary antibody 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.
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Restrictions:	For Research Use only
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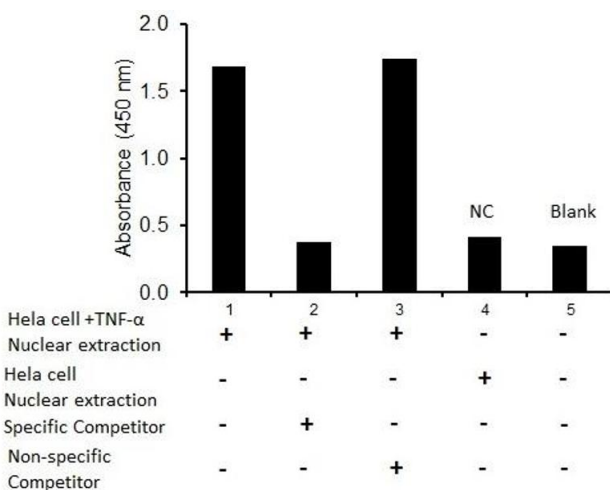
Handling

Storage:	-20 $^{\circ}$ C
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Storage Comment:	Upon receipt, the positive control should be removed and stored at -20 $^{\circ}$ or -80 $^{\circ}$ C. The remainder of the kit can be stored for up to 6 months at 2-8 $^{\circ}$ C from the date of shipment. Opened Microplate Wells or reagents may be stored for up to 1 month at 2 $^{\circ}$ to 8 $^{\circ}$ C. Return unused wells to the pouch containing desiccant pack, reseal along entire edge. Note: The kit can be used within one year if the whole kit is stored at -20 $^{\circ}$ C upon receipt. Avoid repeated freeze-thaw cycles.
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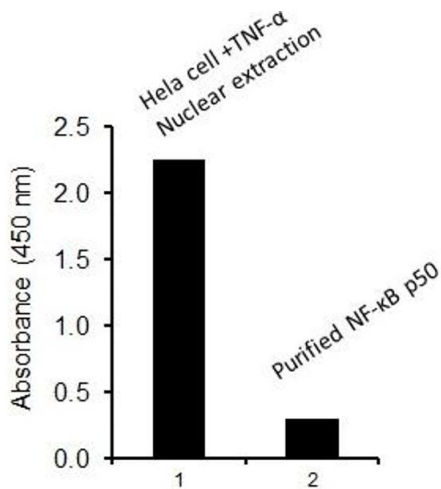
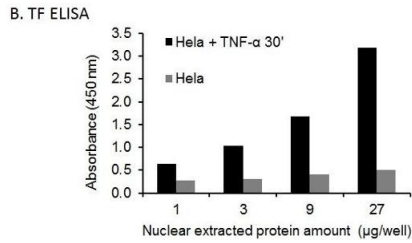
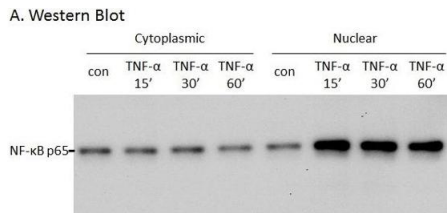
Expiry Date:	6 months
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Images



Activity Assay

Image 1. Transcription factor activity assay of NF- κ B p65 from nuclear extracts of HeLa cells or HeLa cells treated with TNF- α with the specific competitor or non-specific competitor. The result shows specific binding of NF- κ B p65 to the NF- κ B DNA binding site.



Activity Assay

Image 2. Transcription factor activity assay of NF- κ B p65 from nuclear extracts of HeLa cells or HeLa cells treated with TNF- α . After stimulation with TNF- α , activated NF- κ B p65 is translocated into the nucleus where it binds with its corresponding DNA.

Activity Assay

Image 3. Transcription factor activity assay of NF- κ B p65 from nuclear extracts of HeLa cells treated with TNF- α and purified NF- κ B p50. No cross-reactivity has been observed between NF- κ B p65 and p50.