

Datasheet for ABIN4889784

**NF- $\kappa$ B p65 ELISA Kit**

3 Images

[Go to Product page](#)

## Overview

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

## Product Details

Purpose:	Human NF- $\kappa$ B p65 Transcription Factor Activity Assay. This assay uses a dsDNA coated plate with canonical NF- $\kappa$ B binding sequences to semi-quantitatively detect active NF- $\kappa$ 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- $\kappa$ B p65 in whole lysates and nuclear extracts.
Characteristics:	<ul style="list-style-type: none"><li>• Specific transcription factor-DNA binding assay</li><li>• Perfect alternative to EMSA</li><li>• Easy to perform in an ELISA format</li><li>• Non-radioactive assay</li><li>• High throughput (96 well plate format)</li><li>• Assay can be completed within 5 hours</li></ul>

## Product Details

Components:	<ul style="list-style-type: none"><li>• 96-well Strip Microplate pre-coated with DNA probes</li><li>• DNA Binding Buffer</li><li>• Positive Control Sample</li><li>• Specific Competitor DNA probe</li><li>• Non-specific Competitor DNA probe</li><li>• Assay Reagent</li><li>• DTT</li><li>• Wash Buffer</li><li>• Primary Antibody</li><li>• HRP-conjugated Secondary Antibody</li><li>• TMB One-Step Substrate Reagent</li><li>• Stop Solution</li></ul>
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Material not included:	<ul style="list-style-type: none"><li>• Distilled or deionized water</li><li>• 100 mL and 1 liter graduated cylinders</li><li>• Tubes to prepare sample dilutions Absorbent paper</li><li>• Precision pipettes to deliver 2 µL to 1 mL volumes</li><li>• Adjustable 1-25 mL pipettes for reagent preparation</li><li>• Benchtop rocker or shaker</li><li>• Microplate reader capable of measuring absorbance at 450 nm</li></ul>
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## Target Details

Target:	NF-kB p65 (NFkBp65)
Alternative Name:	NFKBP65 ( <a href="#">NFkBp65 Products</a> )
Gene ID:	5970
UniProt:	<a href="#">Q04206</a>
Pathways:	<a href="#">NF-kappaB Signaling</a> , <a href="#">RTK Signaling</a> , <a href="#">TCR Signaling</a> , <a href="#">TLR Signaling</a> , <a href="#">Fc-epsilon Receptor Signaling Pathway</a> , <a href="#">Neurotrophin Signaling Pathway</a> , <a href="#">Activation of Innate immune Response</a> , <a href="#">Cellular Response to Molecule of Bacterial Origin</a> , <a href="#">Hepatitis C</a> , <a href="#">Toll-Like Receptors Cascades</a> , <a href="#">S100 Proteins</a>

## Application Details

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

## Application Details

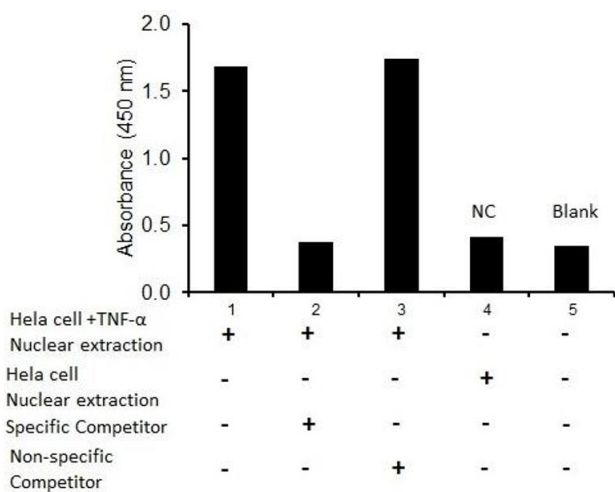
Protocol:	<ol style="list-style-type: none"><li>1. Prepare all reagents and samples as instructed in the manual.</li><li>2. Add 100 µL of sample or positive control to each well.</li><li>3. Incubate 2 h at RT or O/N at 4 °C.</li><li>4. Add 100 µL of prepared primary antibody to each well.</li><li>5. Incubate 1 h at RT.</li><li>6. Add 100 µL of prepared HRP-secondary antibody to each well.</li><li>7. Incubate 1 h at RT.</li><li>8. Add 100 µL of TMB One-Step Substrate Reagent to each well.</li><li>9. Incubate 30 min at RT.</li><li>10. Add 50 µL of Stop Solution to each well.</li><li>11. Read at 450 nm immediately.</li></ol>
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Restrictions:	For Research Use only
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## Handling

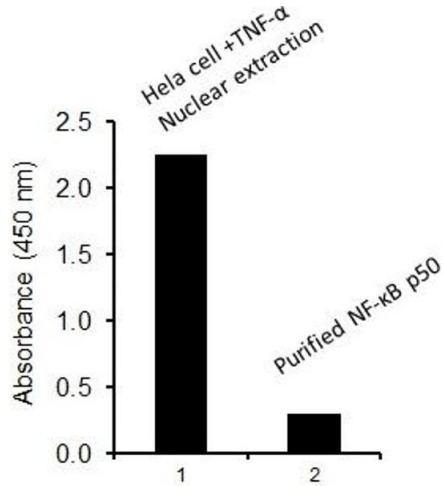
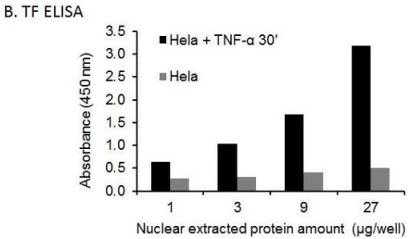
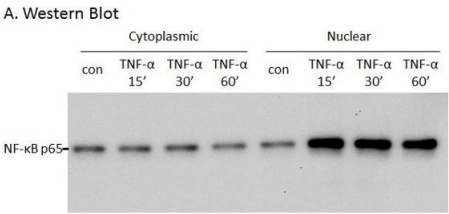
Storage:	-20 °C
Storage Comment:	Upon receipt, the positive control should be removed and stored at -20° or -80°C. The remainder of the kit can be stored for up to 6 months at 2-8°C from the date of shipment. Opened Microplate Wells or reagents may be stored for up to 1 month at 2° to 8°C. Return unused 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°C upon receipt. Avoid repeated freeze-thaw cycles.
Expiry Date:	6 months

## Images



### Activity Assay

**Image 1.** Transcription factor activity assay of NF-κB 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-κB p65 to the NF-κB DNA binding site.



Activity Assay

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

Activity Assay

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