abcam

Product datasheet

NF kappaB p65 (pS536 + Total) ELISA Kit ab176663

SimpleStep ELISA

22 References 5 Images

Overview

Product name NF kappaB p65 (pS536 + Total) ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
(pS536)	6			3.4%
(Total)	6			2.8%

Inter-assay

Sample	n	Mean	SD	CV%
(pS536)	3			10.3%
(Total)	3			8.7%

Sample type Cell Lysate, Tissue Homogenate

Assay type Semi-quantitative

Sensitivity 500 pg/ml

Assay time 1h 30m

Assay duration One step assay

Species reactivity **Reacts with:** Mouse, Human

Predicted to work with: Rat

Product overview Abcam's NFkB p65 (pS536) and NFkB p65 (Total) *in vitro* SimpleStep ELISA™ (Enzyme-Linked Immunosorbent Assay) kit is designed for the semi-quantitative measurement of NFkB p65 (pS536) and Total NFkB p65 protein in Human and mouse cells.

The SimpleStep ELISA™ employs an affinity tag labeled capture antibody and a reporter conjugated detector antibody which immunocapture the sample analyte in solution. This entire complex (capture antibody/analyte/detector antibody) is in turn immobilized via immunoaffinity of an anti-tag antibody coating the well. To perform the assay, samples or standards are added to

the wells, followed by the antibody mix. After incubation, the wells are washed to remove unbound material. TMB substrate is added and during incubation is catalyzed by HRP, generating blue coloration. This reaction is then stopped by addition of Stop Solution completing any color change from blue to yellow. Signal is generated proportionally to the amount of bound analyte and the intensity is measured at 450 nm. Optionally, instead of the endpoint reading, development of TMB can be recorded kinetically at 600 nm.

As of October 2019, this kit was reformulated with new antibodies to maintain continued long term supply.

Notes

Estimated sensitivity: Phospho-NF-κB p65 (Ser536): 5 µg/mL (tested with HeLa), Total NF-κB p65: 5 µg/mL (tested with HeLa)
Range: Phospho-NF-κB p65 (Ser536): 6-600 µg/mL, Total NF-κB p65: 6-600 µg/mL

Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.
It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

Platform

Microplate

Properties

Storage instructions

Store at +4°C. Please refer to protocols.

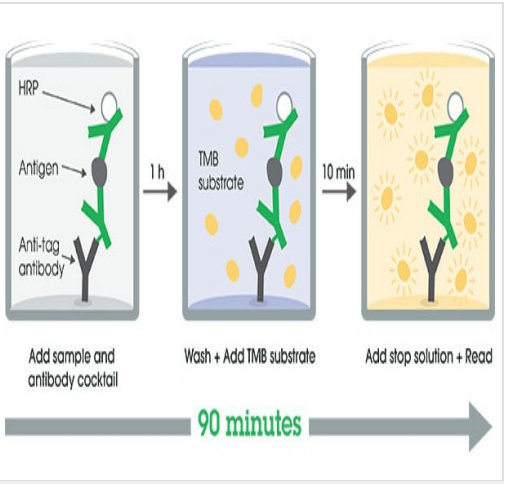
Components	1 x 96 tests	1 x 96 tests
10X Wash Buffer PT	1 x 15ml	1 x 15ml
50X Cell Extraction Enhancer Solution	1 x 1ml	1 x 1ml
5X Cell Extraction Buffer PTR	1 x 12ml	1 x 12ml
Lyophilized NF kappaB p65 Control Lysate	1 vial	1 vial
NF kappaB p65 (pS536) Capture Antibody	1 x 1.5ml	1 x 1.5ml
NF kappaB p65 (pS536) Detector Antibody	1 x 1.5ml	1 x 1.5ml
NF kappaB p65 (Total) Capture Antibody	1 x 1.5ml	1 x 1.5ml
NF kappaB p65 (Total) Detector Antibody	1 x 1.5ml	1 x 1.5ml
Plate Seal	1 unit	1 unit
SimpleStep Pre-Coated 96-Well Microplate (ab206978)	1 unit	1 unit
Stop Solution	1 x 12ml	1 x 12ml
TMB Substrate	1 x 12ml	1 x 12ml

Cellular localization

NF-kB p65: Nucleus. Cytoplasm. Nuclear, but also found in the cytoplasm in an inactive form complexed to an inhibitor (I-kappa-B). Colocalized with RELA in the nucleus upon TNF-alpha

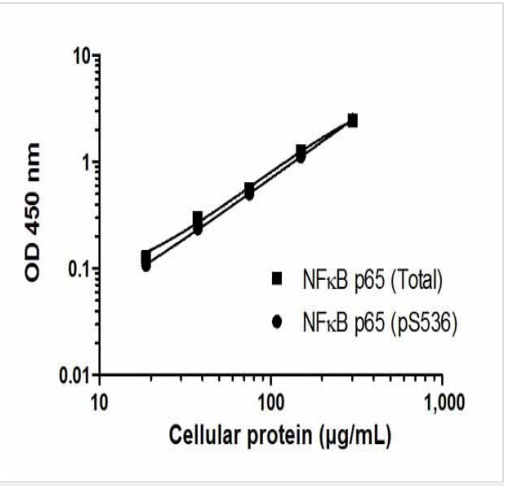
induction. NF-kappaB p65 (Total): Nucleus. Cytoplasm. Nuclear, but also found in the cytoplasm in an inactive form complexed to an inhibitor (I-kappa-B). Colocalized with RELA in the nucleus upon TNF-alpha induction.

Images



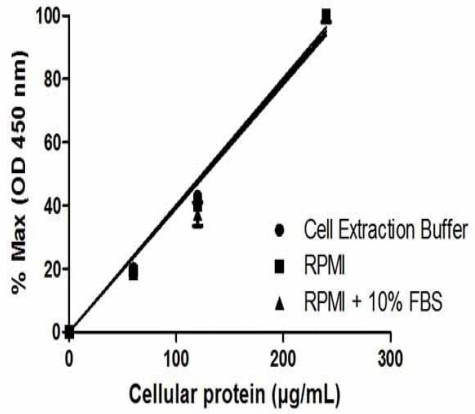
Other - NF kappaB p65 (pS536 + Total) ELISA Kit (ab176663)

SimpleStep ELISA technology allows the formation of the antibody-antigen complex in one single step, reducing assay time to 90 minutes. Add samples or standards and antibody mix to wells all at once, incubate, wash, and add your final substrate. See protocol for a detailed step-by-step guide.



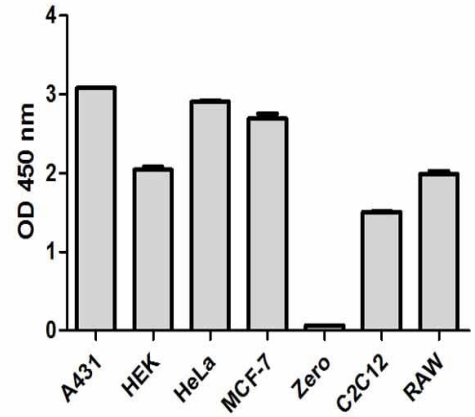
Typical cell lysate dilution series

Example of a typical NFκB p65 (pS536) and NFκB p65 (Total) cell lysate dilution series. Raw data values are shown in the table. Background-subtracted data values (mean +/- SD) are graphed.



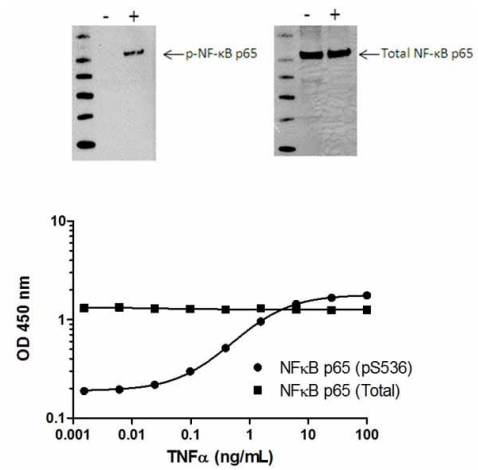
Linearity of dilution

Linearity of dilution in representative sample matrices. Cellular lysates were prepared at 3 concentrations in common media containing 1X Cell Extraction Buffer PTR. Data from duplicate measurements of NFκB p65 (pS536) are normalized and plotted.



Comparison of NFkB p65 expression in different cell lines

Cell line analysis for Total NFκB p65 from 200 µg/mL preparations of cell extracts. Data from triplicate measurements (mean +/- SD) are plotted and compared to 1X Cell Extraction Buffer PTR (zero).



NFkB p65 (pS536) phosphorylation in response to TNFa treatment

Induction of NFκB p65 (pS536) phosphorylation in MCF-7 cells in response to TNFα treatment. HeLa cells were cultured in 96-well tissue culture plates, and treated (10 min) with a dose-range of TNFα before cell lysis. Data from quadruplicate measurements of NFκB p65 (pS536) are plotted and compared against total NFκB p65 protein levels. Comparative NFκB p65 (pS536) and NFκB p65 (Total) data also shown by Western Blot.

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