abcam

Product datasheet

NF kappaB p65 ELISA Kit ab176648

SimpleStep ELISA®

22 References 6 Images

Overview

Product name NF kappaB p65 ELISA Kit

Detection method Colorimetric

Precision

Intra-assay

Sample	n	Mean	SD	CV%
HeLa extract	6			2.8%

Inter-assay

Sample	n	Mean	SD	CV%
HeLa extract	3			8.7%

Sample type Cell Lysate, Tissue Homogenate

Assay type Semi-quantitative

Sensitivity 5 µg/ml

Range 6 µg/ml - 600 µg/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 NFκB p65 *in vitro* SimpleStep ELISA™ (Enzyme-Linked Immunosorbent Assay) kit is designed for the semi-quantitative measurement of NFκB 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

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.

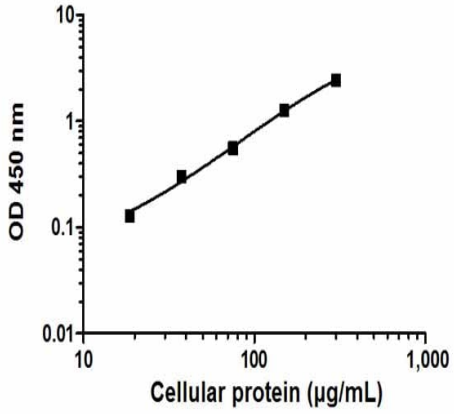
Components	1 x 96 tests	1 x 96 tests
10X Wash Buffer PT	1 x 15ml	1 x 15ml
50X Cell Extraction Enhancer Solution	1 x 1ml	1 x 1ml
5X Cell Extraction Buffer PTR	1 x 12ml	1 x 12ml
Lyophilized NF kappaB p65 Control Lysate	1 vial	1 vial
NF kappaB p65 (Total) Capture Antibody	1 x 3ml	1 x 3ml
NF kappaB p65 (Total) Detector Antibody	1 x 3ml	1 x 3ml
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

Function

NF-kappa-B is a pleiotropic transcription factor which is present in almost all cell types and is involved in many biological processed such as inflammation, immunity, differentiation, cell growth, tumorigenesis and apoptosis. NF-kappa-B is a homo- or heterodimeric complex formed by the Rel-like domain-containing proteins RELA/p65, RELB, NFKB1/p105, NFKB1/p50, REL and NFKB2/p52 and the heterodimeric p65-p50 complex appears to be most abundant one. The dimers bind at kappa-B sites in the DNA of their target genes and the individual dimers have distinct preferences for different kappa-B sites that they can bind with distinguishable affinity and specificity. Different dimer combinations act as transcriptional activators or repressors, respectively. NF-kappa-B is controlled by various mechanisms of post-translational modification and subcellular compartmentalization as well as by interactions with other cofactors or corepressors. NF-kappa-B complexes are held in the cytoplasm in an inactive state complexed with members of the NF-kappa-B inhibitor (I-kappa-B) family. In a conventional activation pathway,

	I-kappa-B is phosphorylated by I-kappa-B kinases (IKKs) in response to different activators, subsequently degraded thus liberating the active NF-kappa-B complex which translocates to the nucleus. NF-kappa-B heterodimeric p65-p50 and p65-c-Rel complexes are transcriptional activators. The NF-kappa-B p65-p65 complex appears to be involved in invasin-mediated activation of IL-8 expression. The inhibitory effect of I-kappa-B upon NF-kappa-B the cytoplasm is exerted primarily through the interaction with p65. p65 shows a weak DNA-binding site which could contribute directly to DNA binding in the NF-kappa-B complex. Associates with chromatin at the NF-kappa-B promoter region via association with DDX1.
Sequence similarities	Contains 1 RHD (Rel-like) domain.
Domain	the 9aaTAD motif is a transactivation domain present in a large number of yeast and animal transcription factors.
Post-translational modifications	Ubiquitinated, leading to its proteasomal degradation. Degradation is required for termination of NF-kappa-B response. Monomethylated at Lys-310 by SETD6. Monomethylation at Lys-310 is recognized by the ANK repeats of EHMT1 and promotes the formation of repressed chromatin at target genes, leading to down-regulation of NF-kappa-B transcription factor activity. Phosphorylation at Ser-311 disrupts the interaction with EHMT1 without preventing monomethylation at Lys-310 and relieves the repression of target genes. Phosphorylation at Ser-311 disrupts the interaction with EHMT1 and promotes transcription factor activity (By similarity). Phosphorylation on Ser-536 stimulates acetylation on Lys-310 and interaction with CBP; the phosphorylated and acetylated forms show enhanced transcriptional activity. Reversibly acetylated; the acetylation seems to be mediated by CBP, the deacetylation by HDAC3. Acetylation at Lys-122 enhances DNA binding and impairs association with NFKBIA. Acetylation at Lys-310 is required for full transcriptional activity in the absence of effects on DNA binding and NFKBIA association. Acetylation can also lower DNA-binding and results in nuclear export. Interaction with BRMS1 promotes deacetylation of 'Lys-310'.
Cellular localization	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.

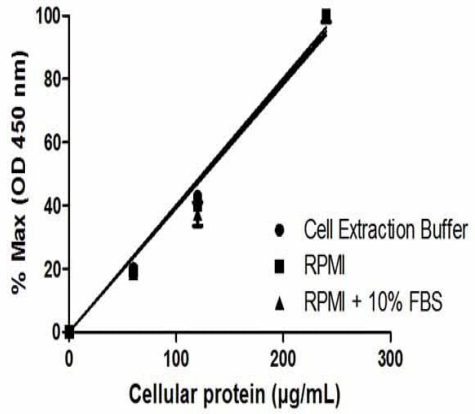
Images



Typical cell lysate dilution series

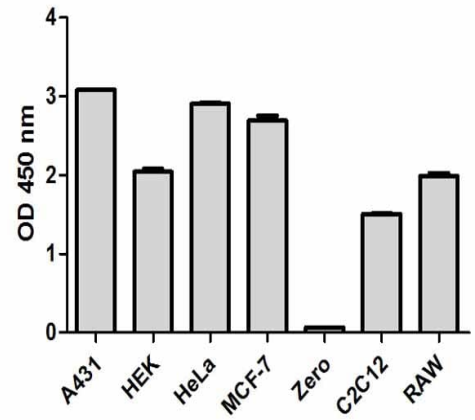
Example of a typical NFκB p65 cell lysate dilution series.

Background-subtracted data values (mean +/- SD) are graphed.



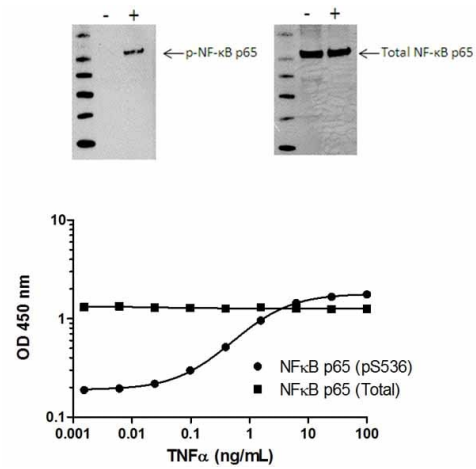
Linearity of dilution

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



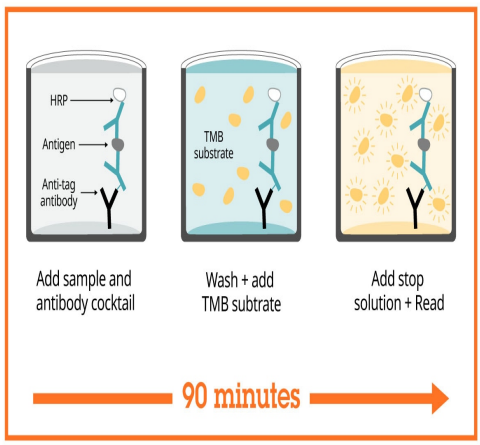
Comparison of NF?B p65 expression in different cell lines

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).



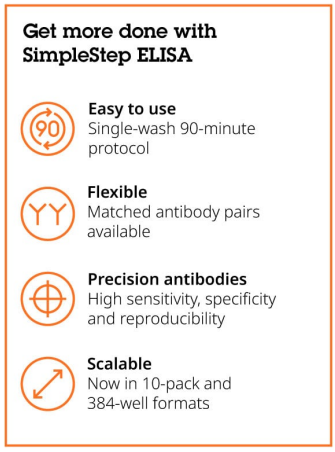
NF?B p65 phosphorylation in response to TNFa treatment

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.



Sandwich ELISA - NF kappaB p65 ELISA Kit (ab176648)

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.



Sandwich ELISA - NF kappaB p65 ELISA Kit (ab176648)

To learn more about the advantages of SimpleStep ELISA® kits see **here**.

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