abcam

Product datasheet

NFkB p65 Transcription Factor Assay Kit ab133112

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Overview

Product name	NFkB p65 Transcription Factor Assay Kit
Detection method	Colorimetric
Sample type	Nuclear Extracts
Assay type	Semi-quantitative
Assay time	3h 30m
Species reactivity	**Reacts with:** Mouse, Rat, Human
Product overview	NFkB p65 Transcription Factor Assay Kit ab133112 is a non-radioactive, sensitive ELISA-based method for detecting specific transcription factor DNA binding activity in nuclear extracts. In the NFkB p65 assay, a double stranded DNA sequence containing the NFkB response element is immobilized onto the bottom of the wells of a 96-well plate. NFkB contained in a nuclear extract, binds to the NFkB response element, and is detected using an anti NFkB p65 antibody. A secondary antibody conjugated to HRP is added to provide a colorimetric readout at 450 nm. NFkB p65 transcription factor assay protocol summary: - prepare nuclear extracts from cells - add samples to wells - incubate for 1 hr or o/n - wash with wash buffer - add NFkB antibody and incubate for 1 hr , then wash - add HRP-conjugated secondary antibody and incubate for 1 hr, then wash - add developing solution and incubate for 15-45 min - add stop solution - analyze with microplate reader
Platform	Microplate reader

Properties

Storage instructions	Please refer to protocols.

Components	96 tests
96-Well Plate Cover	1 unit
Polysorbate 20	1 vial
Transcription Factor Antibody Binding Buffer (10X)	1 x 3ml
Transcription Factor Binding Assay Buffer (4X)	1 x 3ml
Transcription Factor Developing Solution	1 x 12ml
Transcription Factor Goat Anti-Rabbit HRP Conjugate	1 x 100µl
Transcription Factor NFkB (Human p65) Positive Control	1 vial
Transcription Factor NFkB (p65) Primary Antibody	1 vial
Transcription Factor NF-kB 96-Well Strip Plate	1 unit
Transcription Factor NFkB Competitor dsDNA	1 vial
Transcription Factor Reagent A	1 x 120µl
Transcription Factor Stop Solution	1 x 12ml
Wash Buffer Concentrate (400X)	1 x 5ml

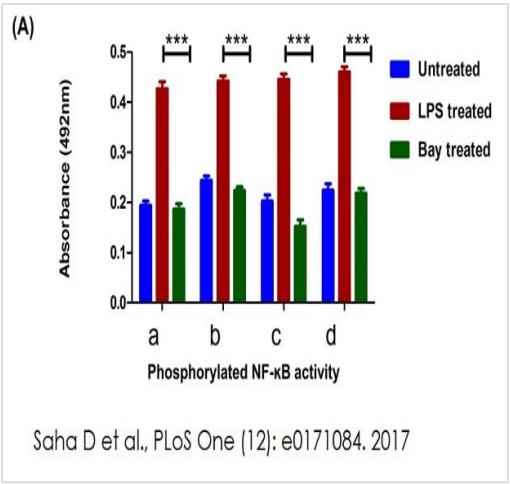
Function NF-kappa-B is a pleiotropic transcription factor which is present in almost all cell types and is involved in many biological processed such as inflammation, immunity, differentiation, cell growth, tumorigenesis and apoptosis. NF-kappa-B is a homo- or heterodimeric complex formed by the Rel-like 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.

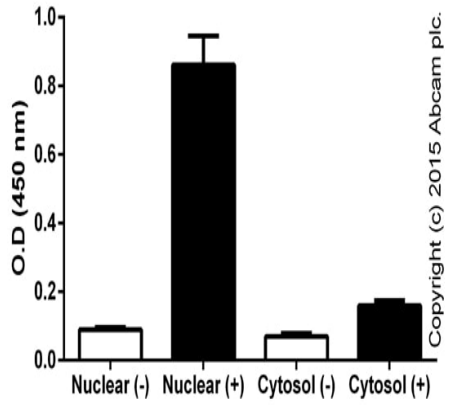
Images



Determining p65-NF-?B by ELISA using ab133112

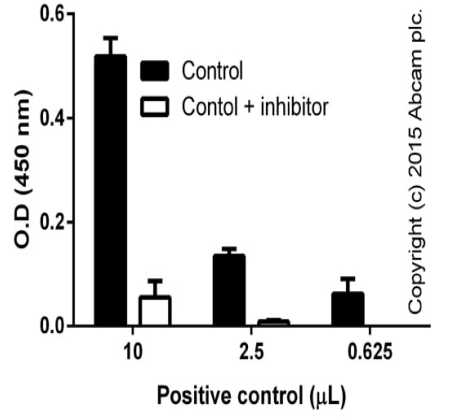
Saha D et al., PLoS One, 12(2). Fig 9a. doi: 10.1371/journal.pone.0171084 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

After the treatment with LPS (10 µg/ml for 6 hrs), cells were lysed with hypotonic HEPES lysis buffer (pH 7.4) and centrifuged at 1000 g for 10 min at 4°C, supernatants were collected and used for the determination of intracellular p65- NF-κB by ELISA. The absorbance was read at 450 nm using spectrophotometer.



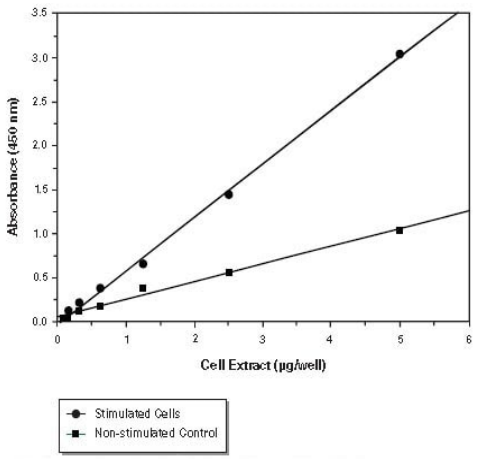
Functional Assay: ab133112 NFkB p65 Transcription Factor Assay Kit

Jurkat cells were treated with PMA and ionomycin (+). Nuclear lysates were extracted (**ab113474**) and 40 uL, corresponding to 4e6 cells, were tested in duplicates (+/- SD).



Functional Assay: ab133112 NFkB p65 Transcription Factor Assay Kit

Titration of positive control with or without inhibitor, background signal subtracted (duplicates; +/- SD).



Functional Studies - NFkB p65 Transcription Factor Assay Kit (ab133112)

Assay of cell lysates isolated from stimulated (20 ng/ml TNF alpha for 30 minutes) and nonstimulated HeLa cells demonstrating NFkB (p65) activity.

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