

NFkB p65 Transcription Factor Assay Kit ab133112

★★★★★ [1 Abreviews](#) [86 References](#) [4 图像](#)

概述

产品名称	NFkB p65 Transcription Factor Assay试剂盒
检测方法	Colorimetric
样品类型	Nuclear Extracts
检测类型	Semi-quantitative
检测时间	3h 30m
种属反应性	与反应: Mouse, Rat, Human
产品概述	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

平台	Microplate reader
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性能

存放说明	Please refer to protocols.
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组件	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 NFκB (Human p65) Positive Control	1 vial
Transcription Factor NFκB (p65) Primary Antibody	1 vial
Transcription Factor NF-κB 96-Well Strip Plate	1 unit
Transcription Factor NFκB 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

功能

NF-kappa-B is a pleiotropic transcription factor which is present in almost all cell types and is involved in many biological processes 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, NFκB1/p105, NFκB1/p50, REL and NFκB2/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 invasion-mediated activation of IL-8 expression. The inhibitory effect of I-kappa-B upon NF-kappa-B in 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.

序列相似性

Contains 1 RHD (Rel-like) domain.

结构域

the 9aaTAD motif is a transactivation domain present in a large number of yeast and animal transcription factors.

翻译后修饰

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.

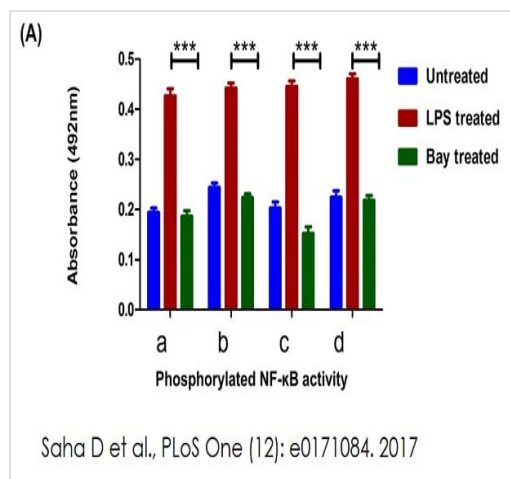
Phosphorylation at Ser-311 disrupts the interaction with EHMT1 and promotes transcription factor activity (By similarity). Phosphorylation on Ser-536 stimulates acetylation on Lys-310 and interaction with CBP; the phosphorylated and acetylated forms show enhanced transcriptional activity.

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'.

细胞定位

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.

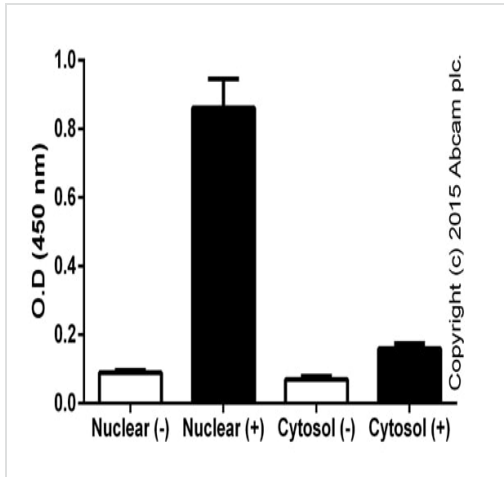
图片



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.

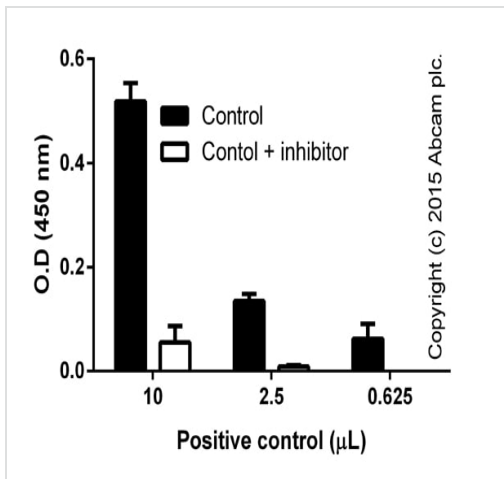
Determining p65-NF-κB by ELISA using ab133112

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/>



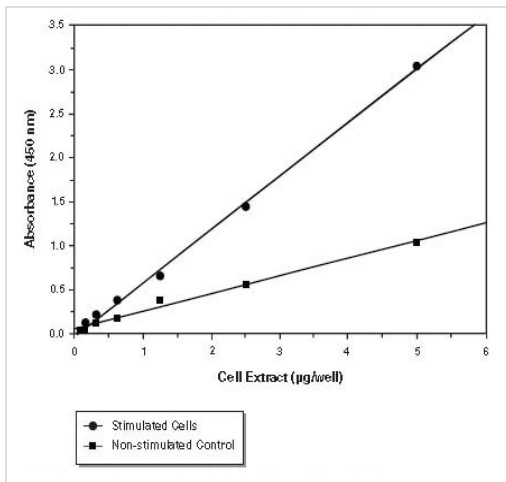
Jurkat cells were treated with PMA and ionomycin (+). Nuclear lysates were extracted ([ab113474](#)) and 40 uL, corresponding to 4e6 cells, were tested in duplicates (+/- SD).

Functional Assay: ab133112 NFkB p65
Transcription Factor Assay Kit



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

Functional Assay: ab133112 NFkB p65
Transcription Factor Assay Kit



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

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

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