abcam

Product datasheet

NFkB p65 Transcription Factor Assay Kit (Colorimetric) ab210613

5 References 2 图像

概述

产品名称	NFkB p65 Transcription Factor Assay试剂盒(Colorimetric)
检测方法	Colorimetric
样品类型	Nuclear Extracts
检测类型	Semi-quantitative
灵敏度	< 500 ng/well
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 (Colorimetric) (ab210613) is a high throughput assay to quantify NFkB p65 activation in nuclear extracts. This assay combines a quick ELISA format with a sensitive and specific non-radioactive assay for transcription factor activation. A specific double stranded DNA sequence containing the NFkB p65 consensus binding site (5' – GGGACTTTCC – 3') has been immobilized onto a 96-well plate. Active NFkB p65 present in the nuclear extract specifically binds to the oligonucleotide. NFkB p65 is detected by a primary antibody that recognizes an epitope of NFkB p65 accessible only when the protein is activated and bound to its target DNA. An HRP-conjugated secondary antibody provides sensitive colorimetric readout that at OD 450 nm. This product detects human, mouse and rat NFkB p65. Key performance and benefits: Assay time: 3.5 hours (cell extracts preparation not included). Detection limit: < 0.5 µg nuclear extract/well. Detection range: 0.2 – 10 µg nuclear or whole cell extract/well.
说明	The transcription factor NFkB is implicated in the regulation of many genes that code for mediators of the immune, acute phase and inflammatory responses. The DNA-binding protein complex recognizes a discrete nucleotide sequence (5´-GGGACTTTCC-3´) in the upstream region of a variety of cellular and viral response genes. NFkB is composed of homo- and

heterodimeric complexes of members of the Rel (NFkB) family. There are five subunits of the NFkB family in mammals: p50, p65 (RelA), c-Rel, p52 and RelB. These proteins share a conserved 300 amino acid sequence in the N-terminal region, known as the Rel homology domain, that mediates DNA binding, protein dimerization and nuclear localization. This domain is also a target of the IkB inhibitors, which include IkBα, IkBβ, IkBγ, Bcl-3, p105 and p100. Various dimer combinations of the NFkB subunits have distinct DNA binding specificities and may serve to activate specific sets of genes such as adhesion molecules, immunoreceptors and cytokines. The p50/p65 (NFkB1/RelA) heterodimers and the p50 homodimers are the most common dimers found in the NFkB signaling pathway. In the majority of cells, NFkB exists in an inactive form in the cytoplasm, bound to the inhibitory IkB proteins. Treatment of cells with various inducers results in the phosphorylation, ubiquitination and subsequent degradation of IkB proteins. Proteolytic cleavage of p105 results in two proteins: p50, which has DNA-binding activity but no transactivation domain, and its antagonist, the inhibitory IkBg protein. This results in the release of NFkB dimers, which subsequently translocate to the nucleus, where they activate appropriate target genes. NFkB can be activated by a number of stimuli, including components of bacterial cell walls, such as lipopolysaccharide, or inflammatory cytokines, such as TNF-α or IL-1β.

平台 Microplate reader

性能

存放说明 Please refer to protocols.

组件	1 x 96 tests	5 x 96 tests
10X Antibody Binding Buffer	1 x 2.2ml	1 x 11ml
10X Wash Buffer	1 x 22ml	1 x 110ml
96-well NFkB assay plate	1 unit	5 units
Anti-rabbit HRP-conjugated IgG	1 x 11µl	1 x 55µl
Binding Buffer	1 x 10ml	1 x 50ml
Developing Solution	1 x 11ml	1 x 55ml
Dithiothreitol (DTT) (1 M)	1 x 100µl	1 x 500µl
Herring sperm DNA	1 x 100µl	1 x 500µl
Lysis Buffer	1 x 10ml	1 x 50ml
Mutated oligonucleotide (10 pmol/µL)	1 x 100µl	1 x 500µl
NFkB p65 antibody	1 x 11µl	1 x 55µl
Plate sealer	1 unit	5 units
Positive control nuclear extract	1 x 40µl	1 x 200µl
Protease Inhibitor Cocktail	1 x 100µl	1 x 500µl

组件	1 x 96 tests	5 x 96 tests
Stop Solution	1 x 11ml	1 x 55ml
Wild-type oligonucleotide (10 pmol/µL)	1 x 100µl	1 x 500µl

功能

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.

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.

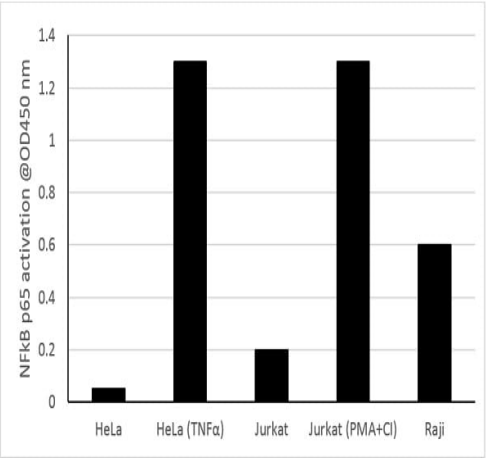
翻译后修饰

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

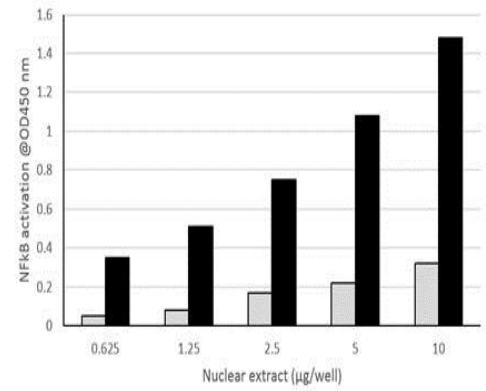
Nucleus. Cytoplasm. Nuclear, but also found in the cytoplasm in an inactive form complexed to an inhibitor (I-kappa-B). Colocalized with RELA in the nucleus upon TNF-alpha induction.

图片



TYPICAL DATA

Nuclear extracts (10 µg/well) prepared from a variety of cell lines were tested for NFkB p65 activity. Cell lines were untreated HeLa cells, HeLa cells treated with TNFα, untreated Jurkat cells, Jurkat cells treated with PMA and calcium ionophore (CI), and Raji cells. These results are provided for demonstration only.



Unstimulated Jurkat (Gray) and stimulated Jurkat (TPA + CI) (Black) cells were tested for NFkB p65 activation.

Different amounts of nuclear extracts from unstimulated Jurkat (white) and stimulated with TPA and calcium ionophore (black) were tested for NFkB p65 activation. This data is provided for demonstration only.

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