abcam

Product datasheet

NFkB p52 Transcription Factor Assay Kit (Colorimetric) ab207219

1 References 1 图像

概述

产品名称	NFkB p52 Transcription Factor Assay试剂盒 (Colorimetric)
检测方法	Colorimetric
样品类型	Cell Lysate, 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	**预测可用于:** Mouse, Human
产品概述	NFkB p52 Transcription Factor Assay Kit (Colorimetric) (ab207219) is a high throughput assay to quantify NFkB p52 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 consensus binding site (5´ - GGGACTTTCC - 3´) has been immobilized onto a 96-well plate. Active NFkB present in nuclear or whole cell extracts specifically binds to the oligonucleotide. NFkB p52 is detected by a primary antibody that recognizes an epitope of NFkB p52 accessible only when NFkB is activated and bound to its target DNA. An HRP-conjugated secondary antibody provides sensitive colorimetric readout at OD 450 nm. This product detects human and mouse NFkB p52. 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 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 IkBγ 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 p52 antibodies	1 x 11µl	1 x 55µl
Plate sealer	1 unit	5 units
Protease Inhibitor Cocktail	1 x 100µl	1 x 500µl

组件	1 x 96 tests	5 x 96 tests
Raji nuclear extract (2.5 µg/µL)	1 x 40µl	1 x 200µl
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. 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. In a non-canonical activation pathway, the MAP3K14-activated CHUK/IKKA homodimer phosphorylates NFKB2/p100 associated with RelB, inducing its proteolytic processing to NFKB2/p52 and the formation of NF-kappa-B RelB-p52 complexes. The NF-kappa-B heterodimeric RelB-p52 complex is a transcriptional activator. The NF-kappa-B p52-p52 homodimer is a transcriptional repressor. NFKB2 appears to have dual functions such as cytoplasmic retention of attached NF-kappa-B proteins by p100 and generation of p52 by a cotranslational processing. The proteasome-mediated process ensures the production of both p52 and p100 and preserves their independent function. p52 binds to the kappa-B consensus sequence 5'-GGRNNYYCC-3', located in the enhancer region of genes involved in immune response and acute phase reactions. p52 and p100 are respectively the minor and major form; the processing of p100 being relatively poor. Isoform p49 is a subunit of the NF-kappa-B protein complex, which stimulates the HIV enhancer in synergy with p65.

疾病相关

Note=A chromosomal aberration involving NFKB2 is found in a case of B-cell non Hodgkin lymphoma (B-NHL). Translocation t(10;14)(q24;q32) with IGHA1. The resulting oncogene is also called Lyt-10C alpha variant.
Note=A chromosomal aberration involving NFKB2 is found in a cutaneous T-cell leukemia (C-TCL) cell line. This rearrangement produces the p80HT gene which encodes for a truncated 80 kDa protein (p80HT).
Note=In B-cell leukemia (B-CLL) cell line, LB40 and EB308, can be found after heterogeneous chromosomal aberrations, such as internal deletions.

序列相似性

Contains 7 ANK repeats.
Contains 1 death domain.
Contains 1 RHD (Rel-like) domain.

结构域

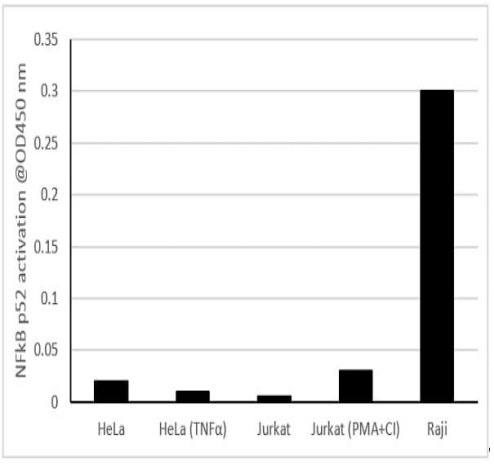
The C-terminus of p100 might be involved in cytoplasmic retention, inhibition of DNA-binding by p52 homodimers, and/or transcription activation.
The glycine-rich region (GRR) appears to be a critical element in the generation of p52.

翻译后修饰

While translation occurs, the particular unfolded structure after the GRR repeat promotes the generation of p52 making it an acceptable substrate for the proteasome. This process is known as cotranslational processing. The processed form is active and the unprocessed form acts as an

	inhibitor (I kappa B-like), being able to form cytosolic complexes with NF-kappa B, trapping it in the cytoplasm. Complete folding of the region downstream of the GRR repeat precludes processing. Subsequent to MAP3K14-dependent serine phosphorylation, p100 polyubiquitination occurs then triggering its proteasome-dependent processing. Constitutive processing is tightly suppressed by its C-terminal processing inhibitory domain, named PID, which contains the death domain.
细胞定位	Nucleus. Cytoplasm. Nuclear, but also found in the cytoplasm in an inactive form complexed to an inhibitor.

图片



TYPICAL DATA

Nuclear extracts (10 µg/well) prepared from a variety of cell lines were tested for NFkB p52 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.

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