

Product datasheet

NFkB p52 Transcription Factor Assay Kit (Colorimetric)
ab207219

1 Image

Overview

Product name	NFkB p52 Transcription Factor Assay Kit (Colorimetric)
Detection method	Colorimetric
Sample type	Cell Lysate, Nuclear Extracts
Assay type	Semi-quantitative
Sensitivity	< 500 ng/well
Assay time	3h 30m
Species reactivity	

Predicted to work with: Mouse, Human 

Product overview

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' - GGGACTTCC - 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.

Notes

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' - GGGACTTCC - 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 (NFκB) family. There are five subunits of the NFκB 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 IκB inhibitors, which include IκBα, IκBβ, IκBγ, Bcl-3, p105 and p100.

Various dimer combinations of the NFκB 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 (NFκB1/RelA) heterodimers and the p50 homodimers are the most common dimers found in the NFκB signaling pathway. In the majority of cells, NFκB exists in an inactive form in the cytoplasm, bound to the inhibitory IκB proteins. Treatment of cells with various inducers results in the phosphorylation, ubiquitination and subsequent degradation of IκB proteins. Proteolytic cleavage of p105 results in two proteins: p50, which has DNA-binding activity but no transactivation domain, and its antagonist, the inhibitory IκBγ protein. This results in the release of NFκB dimers, which subsequently translocate to the nucleus, where they activate appropriate target genes. NFκB 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.

Platform Microplate reader

Properties

Storage instructions Please refer to protocols.

Components	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 NFκB assay plate	1 unit	5 units
Anti-rabbit HRP-conjugated IgG (0.25 µg/µL)	1 x 11 µl	1 x 55 µl
Binding Buffer	1 x 10ml	1 x 50ml
Developing Solution	1 x 11ml	1 x 55ml
Dithiothreitol (DTT) (1 M)	1 x 100 µl	1 x 500 µl
Herring sperm DNA (1 µg/µL)	1 x 100 µl	1 x 500 µl
Lysis Buffer	1 x 10ml	1 x 50ml
Mutated oligonucleotide (10 pmol/µL)	1 x 100 µl	1 x 500 µl
NFκB p52 antibodies	1 x 11 µl	1 x 55 µl
Plate sealer	1 unit	5 units
Protease Inhibitor Cocktail	1 x 100 µl	1 x 500 µl
Raji nuclear extract (2.5 µg/µL)	1 x 40 µl	1 x 200 µl

Components	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

Function

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, 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.

Involvement in disease

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 Lyl-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.

Sequence similarities

Contains 7 ANK repeats.

Contains 1 death domain.

Contains 1 RHD (Rel-like) domain.

Domain

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.

Post-translational modifications

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.

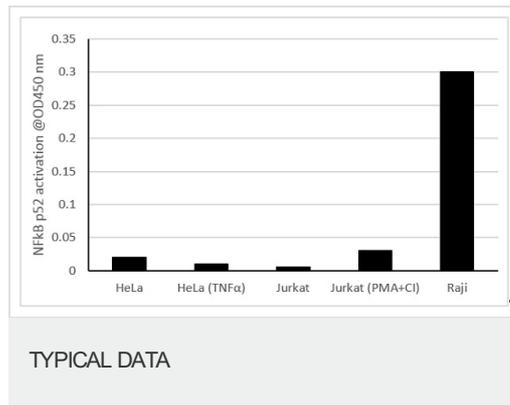
Subsequent to MAP3K14-dependent serine phosphorylation, p100 polyubiquitination occurs then triggering its proteasome-dependent processing.

Constitutive processing is tightly suppressed by its C-terminal processing inhibitory domain, named PID, which contains the death domain.

Cellular localization

Nucleus. Cytoplasm. Nuclear, but also found in the cytoplasm in an inactive form complexed to an inhibitor.

Images



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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