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Product datasheet

Human NFkB p50 Transcription Factor Assay Kit ab133105

1 Abreviews 1 Image

Overview

Product name	Human NFkB p50 Transcription Factor Assay Kit
Detection method	Colorimetric
Sample type	Cell culture extracts, Cell Lysate
Assay type	Semi-quantitative
Species reactivity	**Reacts with:** Human
Product overview	ab133105 Human NFkB p50 Transcription Factor Assay Kit is a non-radioactive, sensitive method for detecting specific transcription factor DNA binding activity in nuclear extracts and whole cell lysates. A 96-well enzyme-linked immunosorbent assay (ELISA) replaces the cumbersome radioactive electrophoretic mobility shift assay (EMSA). A specific double stranded DNA (dsDNA) sequence containing the NFkB response element is immobilized onto the bottom of wells of a 96-well plate. NFkB contained in a nuclear extract, binds specifically to the NFkB response element. NFkB (p50) is detected by addition of specific primary antibody directed against NFkB (p50). A secondary antibody conjugated to HRP is added to provide a sensitive colorimetric readout at 450 nm. ab133105 detects Human NFkB (p50). It will not cross-react with NFkB (p65).
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

Components	96 tests
Transcription Factor Developing Solution	1 x 12ml
Transcription Factor Goat Anti-Rabbit HRP Conjugate	1 x 100µl
Transcription Factor NFkB (Human p50) Positive Control	1 vial
Transcription Factor NFkB (Human p50) 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 RelB-p50 complexes are transcriptional activators. The NF-kappa-B p50-p50 homodimer is a transcriptional repressor, but can act as a transcriptional activator when associated with BCL3. NFKB1 appears to have dual functions such as cytoplasmic retention of attached NF-kappa-B proteins by p105 and generation of p50 by a cotranslational processing. The proteasome-mediated process ensures the production of both p50 and p105 and preserves their independent function, although processing of NFKB1/p105 also appears to occur post-translationally. p50 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. In a complex with MAP3K8, NFKB1/p105 represses MAP3K8-induced MAPK signaling; active MAP3K8 is released by proteasome-dependent degradation of NFKB1/p105.

Sequence similarities

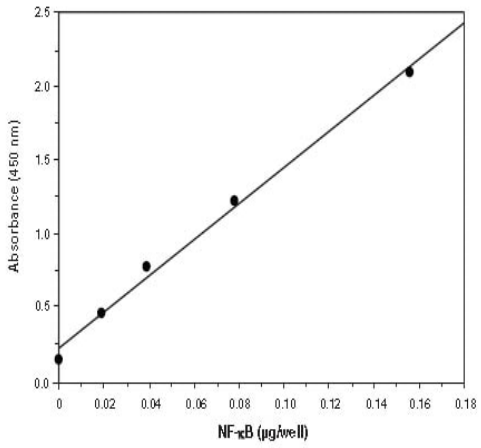
Contains 7 ANK repeats.
Contains 1 death domain.
Contains 1 RHD (Rel-like) domain.

Domain

The C-terminus of p105 might be involved in cytoplasmic retention, inhibition of DNA-binding, and transcription activation.
Glycine-rich region (GRR) appears to be a critical element in the generation of p50.

Post-translational modifications	While translation occurs, the particular unfolded structure after the GRR repeat promotes the generation of p50 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. Phosphorylation at 'Ser-903' and 'Ser-907' primes p105 for proteolytic processing in response to TNF-alpha stimulation. Phosphorylation at 'Ser-927' and 'Ser-932' are required for BTRC/BTRCP-mediated proteolysis. Polyubiquitination seems to allow p105 processing. S-nitrosylation of Cys-61 affects DNA binding.
Cellular localization	Nucleus. Cytoplasm. Nuclear, but also found in the cytoplasm in an inactive form complexed to an inhibitor.

Images



Functional Studies - NFkB p50 Transcription Factor Assay Kit (ab133105)

Sensitivity of the NFkB assay using purified Human recombinant p50.

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