Datasheet for ABIN105291

anti-NF-kB p65 antibody (N-Term)

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Overview

Quantity:	100 µg
Target:	NF-kB p65 (NFkBP65)
Binding Specificity:	N-Term
Reactivity:	Human
Host:	Rabbit
Clonality:	Polyclonal
Application:	Western Blotting (WB), Immunohistochemistry (IHC), ELISA, Immunofluorescence (IF), Gel Shift (GS), Fluorescence Microscopy (FM)

Product Details

Purpose:	NFkB p65 Antibody
Immunogen:	Immunogen: This antibody was purified from whole rabbit serum prepared by repeated immunizations with the NFkB p65 peptide corresponding to the NLS of the human protein conjugated to KLH using maleimide. A residue of cysteine was added to the amino terminal end to facilitate coupling. Immunogen Type: Conjugated Peptide
Isotype:	IgG
Cross-Reactivity (Details):	This affinity-purified antibody is directed against the nuclear localization sequence (NLS) NLS of human p65 and is useful in determining its presence in various assays. The epitope recognized overlaps the NLS of the p65 subunit of the NFkB heterodimer.
Characteristics:	Synonyms: rabbit anti-NFKB p65 antibody, rabbit anti-p65 antibody, rabbit anti-NLS specific

Product Details

	antibody, nuclear localization sequence, NFKB, NFkß, NF-kB, NF-kappaB, NFkappaB, Transcription factor p65, Nuclear factor NF-kappa-B p65 subunit, Nuclear factor of kappa light polypeptide gene enhancer in B-cells 3, RELA, NFKB3
Purification:	Affinity purified antibody
Sterility:	Sterile filtered

Target Details

Target:	NF-kB p65 (NFkBP65)
Alternative Name:	RELA (NFkBP65 Products)
Background:	Background: NFkB was originally identified as a factor that binds to the immunoglobulin kappa light chain enhancer in B cells. It was subsequently found in non-B cells in an inactive cytoplasmic form consisting of NFkB bound to IkB. NFkB was originally identified as a heterodimeric DNA binding protein complex consisting of p65 (RelA) and p50 (NFKB1) subunits. Other identified subunits include p52 (NFKB2), c-Rel, and RelB. The p65, cRel, and RelB subunits are responsible for transactivation. The p50 and p52 subunits possess DNA binding activity but limited ability to transactivate. p52 has been reported to form transcriptionally active heterodimers with the NFkB subunit p65, similar to p50/p65 heterodimers. Low levels of p52 and p50 homodimers can also exist in cells. The heterodimers of p52/p65 and p50/p65 are regulated by physical inactivation in the cytoplasm by IkB-a. IkB-a binds to the p65 subunit, preventing nuclear localization and DNA binding. IkB-a binding masks the NFkB nuclear localization signal (NLS). A broad range of external stimuli lead to activation of NFkB and set off signaling cascades that ultimately converge on the IkB kinase (IKK) complex. Activated IKK specifically and directly phosphorylates IkB-a and this phosphorylation event targets IkB-a for degradation. As a consequence, NFkB NLS is uncovered and nuclear translocation occurs.
Gene ID:	5970, 223468676
UniProt:	Q04206
Pathways:	NF-kappaB Signaling, RTK Signaling, TCR Signaling, TLR Signaling, Fc-epsilon Receptor Signaling Pathway, Neurotrophin Signaling Pathway, Activation of Innate immune Response, Cellular Response to Molecule of Bacterial Origin, Hepatitis C, Toll-Like Receptors Cascades, S100 Proteins

Application Details

Application Notes:	Immunohistochemistry Dilution: 1:200 Gel Shift Dilution: 0.5 µL - 1.0 µL Application Note: Anti-NFKB p65 antibody has been tested in ELISA, ICC, WB, and IF. NFkB gel shift assays are assembled in 20 µL reactions containing 0.28 pmoles NFkB oligo in 10 mM Tris (pH 7.6), 50 mM NaCl, 0.5 mM EDTA, 1.0 mM DTT, 10 % glycerol. Some procedures specify the addition of 0.05 % NP-40. When using purified protein, 250-300 ng should be sufficient to produce a gel shifted complex, while 10 µg HeLa nuclear extract is utilized. The gel shift reactions are then incubated at room temperature for 30 minutes. The complexes are resolved on Tris-Glycine acrylamide gels. Loading dye containing bromophenol blue and xylene cyanol should be added to the negative control reaction only, as these dyes can increase the dissociation of the NFkB complexes. When using HeLa nuclear extract as the source of binding proteins, two sequence-specific gel-shifted complexes are expected, consisting of p50/p50 homodimers and p50/p65 heterodimers. For cells expressing p52, p50, and p65, as many as four sequence-specific gel-shifted complexes could be observed (p52/p52, p50/p50, p52/p65, p50/p65), and if high levels of p65 are present, the p65/p65 homodimer may also be weakly detected. The following reagents have been observed to enhance NFkB binding in vitro: millimolar amounts of GTP and ATP, spermine, spermidine, barium or calcium ions, and µM amounts of Co+3(NH3)6. Western Blot Dilution: 1:2,000 ELISA Dilution: 1:5,000 - 1:25,000 IF Microscopy Dilution: 1:200 Other: User Optimized
Restrictions:	For Research Use only

Handling

Format:	Liquid
Concentration:	1.0 mg/mL
Buffer:	Buffer: 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2 Stabilizer: None Preservative: 0.01 % (w/v) Sodium Azide
Preservative:	Sodium azide
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which should be handled by trained staff only.

Handling

Storage:	4 °C,-20 °C
Storage Comment:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiry Date:	12 months

Publications

Product cited in:

Yu, Chang, Liu, Li, Kevork, Al-Hezaimi, Graves, Park, Wang: "Wnt4 signaling prevents skeletal aging and inflammation by inhibiting nuclear factor-κB." in: **Nature medicine**, Vol. 20, Issue 9, pp. 1009-17, (2014) (PubMed).

Graff, Ettayebi, Hardy: "Rotavirus NSP1 inhibits NFkappaB activation by inducing proteasome-dependent degradation of beta-TrCP: a novel mechanism of IFN antagonism." in: **PLoS pathogens**, Vol. 5, Issue 1, pp. e1000280, (2009) (PubMed).

Lord, Savitsky, Sitcheran, Calame, Wright, Ting, Williams: "Blimp-1/PRDM1 mediates transcriptional suppression of the NLR gene NLRP12/Monarch-1." in: **Journal of immunology (Baltimore, Md. : 1950)**, Vol. 182, Issue 5, pp. 2948-58, (2009) (PubMed).

Liu, Ju, Willmarth, Casimiro, Ojeifo, Sakamaki, Katiyar, Jiao, Popov, Yu, Wu, Joyce, Wang, Pestell: "Nuclear factor-kappaB enhances ErbB2-induced mammary tumorigenesis and neoangiogenesis in vivo." in: **The American journal of pathology**, Vol. 174, Issue 5, pp. 1910-20, (2009) (PubMed).

Sivaramakrishnan, Niranjali Devaraj: "Morin regulates the expression of NF-kappaB-p65, COX-2 and matrix metalloproteinases in diethylnitrosamine induced rat hepatocellular carcinoma." in: **Chemico-biological interactions**, Vol. 180, Issue 3, pp. 353-9, (2009) (PubMed).

There are more publications referencing this product on: Product page

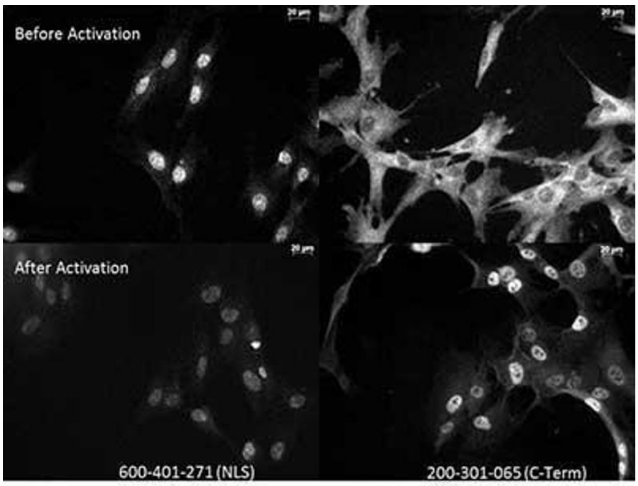
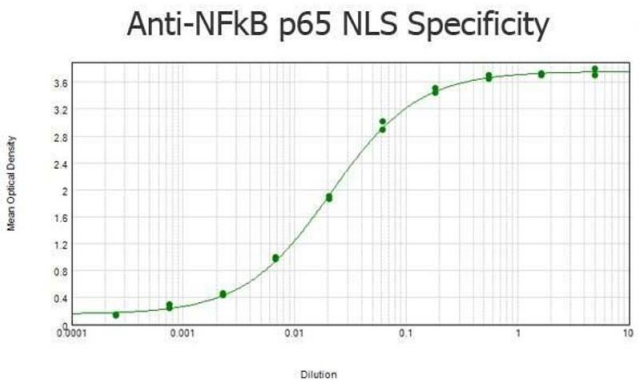


Image 1. Anti NFkB monoclonal antibody – Immunocytochemistry Tissue: Human Fibroblasts Top: Before activation Bottom: After activation with poly IC Left: anti p65 NLS specific lot 18372 Right: 200-301-065 Monoclonal antibody C-Term The two antibodies that are shown target different regions of the p65 protein. The different staining patterns are thought to correspond with different functional regions of the protein.



ELISA

Image 2. ELISA results of purified Rabbit anti-NFkB p65 NLS Specific Antibody tested against BSA-conjugated peptide of immunizing peptide. Each well was coated in duplicate with 0.1µg of conjugate. The starting dilution of antibody was 5µg/ml and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. Assay performed using 3% fish gel, Goat anti-Rabbit IgG Antibody Peroxidase Conjugated (Min X Bv Ch Gt GP Ham Hs Hu Ms Rt & Sh Serum Proteins) and TMB ELISA Peroxidase Substrate .