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Datasheet for 100-4165S

NFKB p65 Antibody

Overview

Description:	Anti-NFKB p65 (Rel A) (RABBIT) Antibody - 100-4165S
Item No.:	100-4165S
Size:	25 µL
Applications:	ELISA, IHC, WB, ChIP, EMSA, IF, IP
Reactivity:	Human, Mouse
Host Species:	Rabbit

Product Details

Background:	Anti NFkB p65 Antibody recognizes NFKB p65 which is a component of NFKB. 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 NFkappaB bound to IkappaB. NFkappaB 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 NFkappaB subunit p65, similar to p50/p65 heterodimers. The heterodimers of p52/p65 and p50/p65 are regulated by physical inactivation in the cytoplasm by IkappaBalpha. Ideal for Cardiovascular , Cell Biology, Immunology, Signal Transduction research.
Synonyms:	rabbit Anti-NFKB p65 antibody, rabbit Anti-Rel A antibody, NFKB, nfkb, NF-kB, NF-kappaB, NFkappaB, Nuclear factor NF-kappa-B p65 subunit
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	Antiserum

Target Details

Gene Name:	RELA
Reactivity:	Human, Mouse

Immunogen Type:	Conjugated Peptide
Immunogen:	NFkB p65 (Rel A) peptide corresponding to a region near the C-terminus of the human protein conjugated to Keyhole Limpet Hemocyanin (KLH).
Purity/Specificity:	This product was prepared from monospecific antiserum by delipidation and defibrination. Anti-NFkB p65 (Rel A) may react non-specifically with other proteins. Control peptide (code #100-4165p) will compete only with the specific reaction of antiserum with the NFkB p65 (Rel A) subunit.
Relevant Links:	• UniProtKB - Q04206 • GeneID - 5970 • NCBI - 223468676

Application Details

Tested Applications:	ELISA, IHC, WB
Suggested Applications:	ChIP, EMSA, IF, IP (Based on references)
Application Note:	Anti-NFkB p65 (Rel A) is tested by immunoblot of human and mouse NFkB p65 (Rel A), immunohistochemistry, and ELISA. This product was also tested in a gel supershift assay, IP, IF, and ChIP and found to be reactive against all p65 (Rel A) containing human, mouse or rat NFkB complexes using 0.5 to 1.0 µl per assay.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ChIP:	1 µl/IP
ELISA:	1:5,000
EMSA:	05 µL - 1.0 µL
IF:	User Optimized
IHC:	1:400
IP:	User Optimized
WB:	1:2,000 - 1:5,000

Formulation

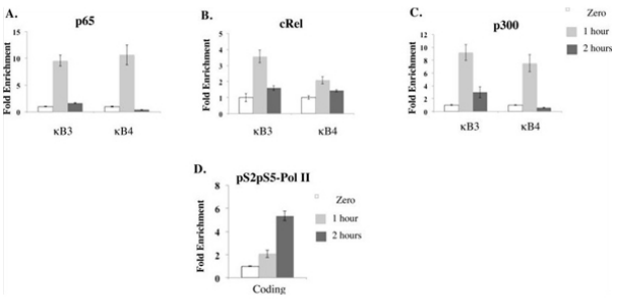
Physical State:	Liquid (sterile filtered)
Concentration:	80 mg/mL by Refractometry
Buffer:	None

Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

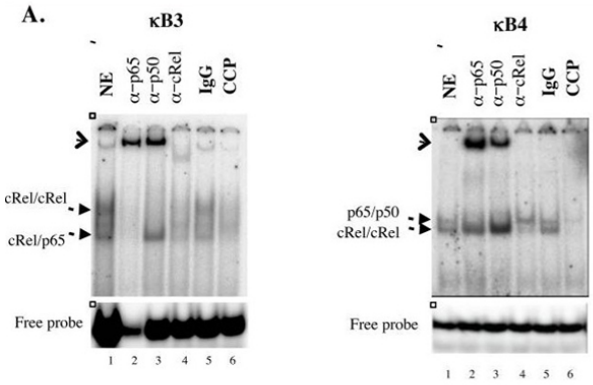
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



ChIP
NF-κB complexes are recruited to the EIF4E promoter and promote transactivation. BJAB cells were stimulated with PMA for 0, 1 or 2 hours and subjected to chromatin immunoprecipitation using antibodies specific for (A) NFKB p65 (p/n 100-4165), (B) cRel, (C) p300 transcription factor (p/n 100-301-176) and phosphorylated Pol II (Ser2/Ser5) (D). Data were normalized to IgG control and represented as fold enrichment with respect to untreated cells. Error bars represent standard deviations from triplicate measurements of a representative experiment. Figure 3. PMID: 23467026

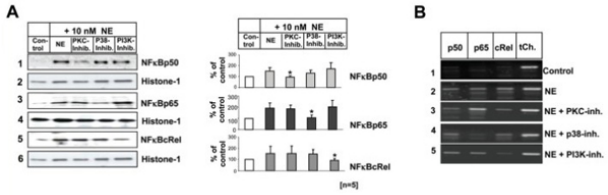


Western Blot

Constitutively active NF-κB regulates eIF4E expression in KM-H2 cells.

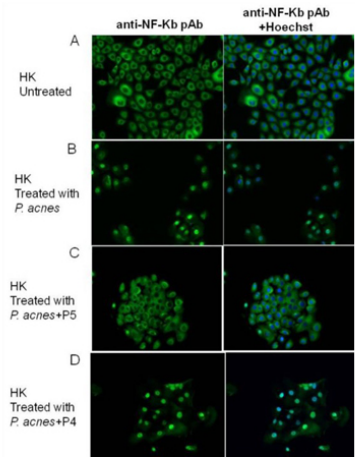
(A) EMSA analysis of KM-H2 nuclear extracts using probes corresponding to the κB3 and κB4 sites. Supershift analysis using antibodies against NFKB p65 (p/n 100-4165), NFKB p50 p/n 100-4164), cRel and IgG control as well as competition with consensus cold probe (CCP) were done. Protein/DNA complexes are indicated by arrows and supershifted complexes by arrowheads. Free probe is also shown.

Figure 5. PMID: 23467026



Western Blot

Western Blot and ChIP results using NF-κB. Effects of PKC, p38MAPK or PI3K pathway inhibition on NF-κB subunits nuclear translocation in vitro: THP-1 cells were preincubated for 45 min in the absence or presence of the PKC, p38MAPK or PI3K inhibitor before treatment with NE for 15 min. (A) Nuclear extracts of THP-1 cells were immunoblotted with antibodies to p50, p65 (p/n 100-4165), cRel (p/n 100-4166) and histone-1. The signal intensity was evaluated and is summarized in the bargraphs. Statistical significant differences are indicated (*$p < 0.05$). Data represent the mean ± SD (n = 5). (B) The recruitment of p50, p65 and cRel-subunits to the ICAM-1 promoter was determined using ChIP-assays. Fig 3. PMID: 23114885.

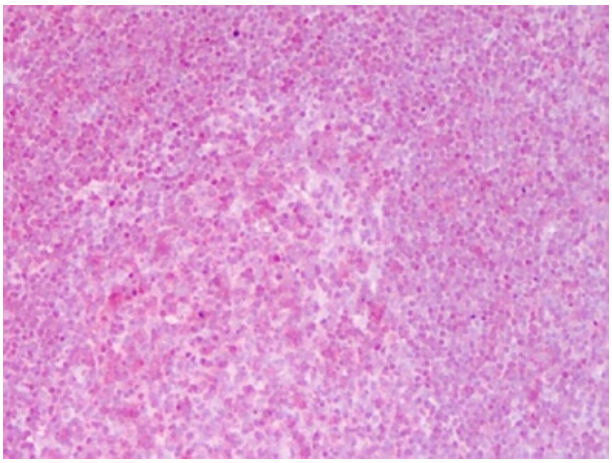


Immunofluorescence Microscopy

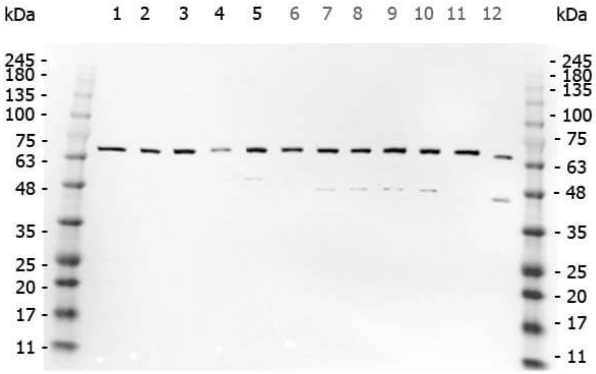
P5 inhibits NF-κB nuclear translocation in P. acnes-infected HK cells.

The intracellular distribution of NF-κB was determined by immunofluorescent labeling of NF-κB p65 (green), while nuclei were Hoechst stained (blue): (A) untreated HK cells; (B) HK cells treated with P. acnes; (C) HK cells treated with P. acnes plus P5; HK cells treated with P. acnes plus P4 (D).

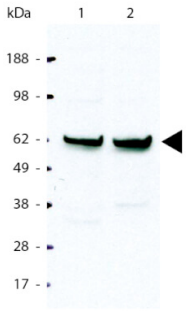
Fig 5. PMID: 26197393.



Immunohistochemistry
Immunohistochemistry of NFKB p65 (Rel A) antibody. Tissue: lymphocytes and germinal center cells of the human tonsil. Fixation: formalin fixed paraffin embedded. Antigen retrieval: user optimized. Primary antibody: NFKB p65 (Rel A) antibody at 1:400. Secondary antibody: Peroxidase goat anti-rabbit at (p/n 611-103-122) 1:10,000 for 45 min at RT. Localization: nuclear and occasionally cytoplasmic. Staining: Moderate positive nuclear or cytoplasmic staining was observed in lymphocytes and germinal center cells of the tonsil.



Western Blot
Western Blot of Rabbit anti-NFkB antibody. Marker: Opal Pre-stained ladder (p/n MB-210-0500). Lane 1: HEK293 lysate (p/n W09-000-365). Lane 2: HeLa Lysate (p/n W09-000-364). Lane 3: MCF-7 Lysate (p/n W09-000-360). Lane 4: Jurkat Lysate (p/n W09-000-370). Lane 5: A431 Lysate (p/n W09-000-361). Lane 6: A549 Lysate (p/n W09-001-372). Lane 7: LNCap Lysate (p/n W09-001-GJ9). Lane 8: MOLT-4 Lysate (p/n W09-001-GK2). Lane 9: Ramos Lysate (p/n W09-000-GK4). Lane 10: Raji Lysate (p/n W09-001-368). Lane 11: A-172 Lysate (p/n W09-001-GL5). Lane 12: NIH/3T3 Lysate (p/n W10-000-358). Load: 10 µg per lane. Primary antibody: NFkB antibody at 1:500 overnight at 4C. Secondary antibody: Peroxidase rabbit secondary antibody (p/n 611-103-122) at 1:30,000 for 60 min at RT. Blocking Buffer: 1% Casein-TTBS (p/n MB-082) for 30 min at RT. Predicted/Observed size: 65 kDa for NFkB.



Western Blot
Western Blot of Rabbit anti-NFkB p65 (Rel A) antibody. Lane 1: HeLa cell lysate (p/n W09-000-364). Lane 2: HeLa cell lysate (p/n W09-000-364). Load: 35 µg per lane. Primary antibody: NFkB p65 Rel A antibody at 1:5000 for 2 H at RT. Secondary antibody: Peroxidase rabbit secondary antibody at 1:2000 for 60 min at RT. Block: 5% BLOTTO 2 H at RT. Predicted/Observed size: ~65 kDa, ~65 kDa for NFkb p65 Rel A. Other band(s): None.

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