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Datasheet for 600-401-271

NFkB p65 Antibody

Overview

Description:	Anti-NFKB p65 NLS specific (RABBIT) Antibody - 600-401-271
Item No.:	600-401-271
Size:	100 µg
Applications:	ELISA, IF, WB, EMSA, IHC
Reactivity:	Human
Host Species:	Rabbit

Product Details

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.
Synonyms:	rabbit anti-NFKB p65 antibody, rabbit anti-p65 antibody, rabbit anti-NLS specific 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
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	RELA
Reactivity:	Human
Immunogen Type:	Conjugated Peptide
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.
Purity/Specificity:	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. Therefore, the antibody selectively binds to the activated form of NFkB. Anti-NFkB p65 NLS may react non-specifically with other proteins.
Relevant Links:	• UniProtKB - Q04206 • NCBI - 223468676 • GeneID - 5970

Application Details

Tested Applications:	ELISA, IF, WB
Suggested Applications:	EMSA, IHC (Based on references)
Application Note:	Anti-NFKB p65 antibody has been tested in ELISA, ICC, WB, and IF. NFkB gel shift assays are assembled in 20µl reactions containing 0.28 pmoles NFkB oligo in 10mM 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 10µg 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.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.

ELISA:	1:5,000 - 1:25,000
EMSA:	0.5 µL - 1.0 µL
IF:	1:200
IHC:	1:200
WB:	1:2,000

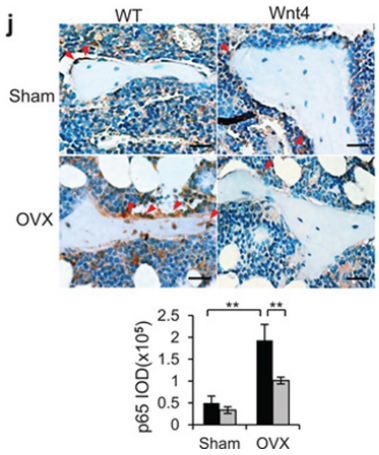
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

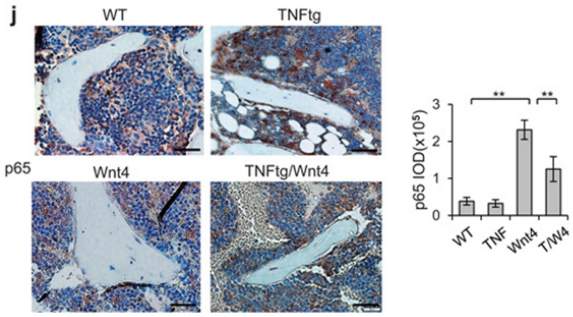
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	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.
Expiration:	Expiration date is one (1) year from date of receipt.

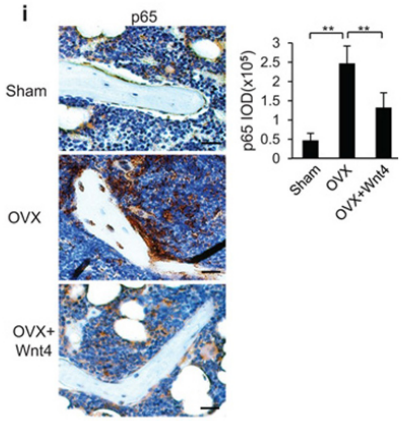
Images



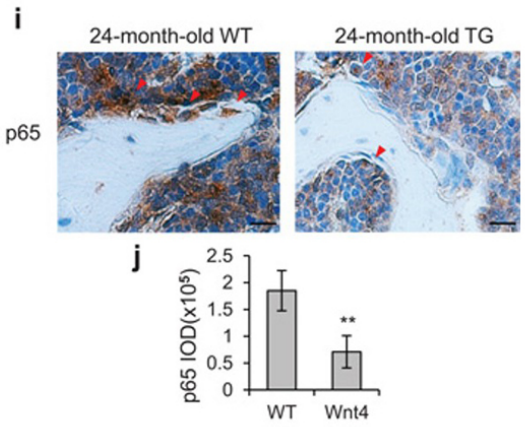
Immunohistochemistry
(j) Immunostaining and quantification of active p65 in trabecular bone cells and surrounding bone marrow cells in WT and Wnt4 mice after OVX or sham operation. Scale bars, 30 µm. IOD, integral optical density. n = 8 for sham groups; n = 12 for OVX groups. *P < 0.05, ** P < 0.01, unpaired two-tailed t-test. Fig 2. PMID: 25108526



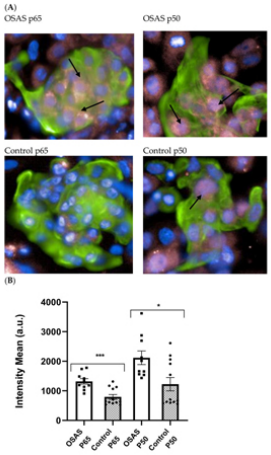
Immunohistochemistry
(j) Immunostaining with anti-active p65 and quantification of NF-κB activity surrounding the trabecular bone in WT, Wnt4, TNFtg and TNFtg/Wnt4 mice. Scale bars, 40µm. TNF, TNFtg mice; T/W4, TNFtg/Wnt4 mice. n = 6 per group for WT and WNT4 mice; n = 8 per group for TNFtg and TNFtg/Wnt mice. *P < 0.05, ** P < 0.01, unpaired two-tailed t-test. Fig 3. PMID: 25108526



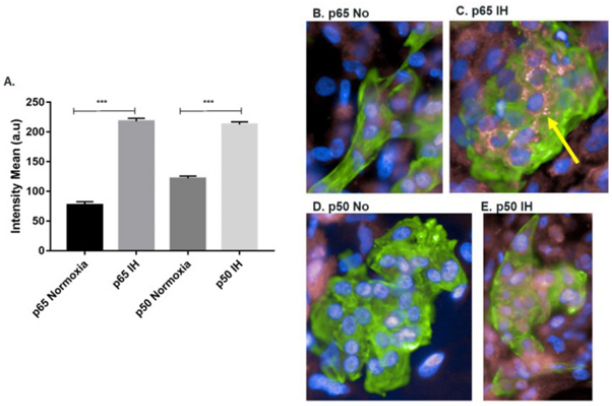
Immunohistochemistry
(i) Immunostaining with anti-active p65 and quantification of NF-κB activity surrounding the trabecular bones from mice after sham operation, OVX and OVX with rWnt4 injection. Scale bars, 30 µm. Fig 6. PMID: 25108526



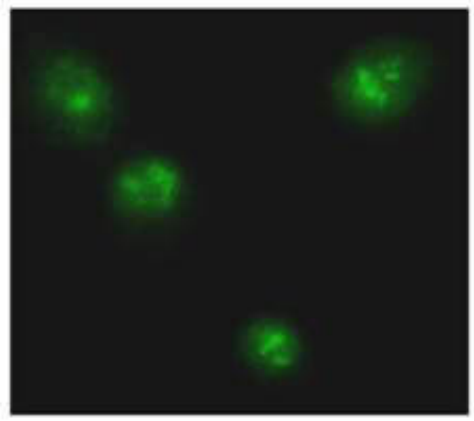
Immunohistochemistry
(i) Immunostaining with anti-active p65 and quantification of NF-κB activity surrounding the trabecular bones from 24-months-old WT and Wnt4 mice. Scale bars, 25 μm. n = 12 mice per group. *P < 0.05, ** P < 0.01, unpaired two-tailed t-test. Fig 4. PMID: 25108526



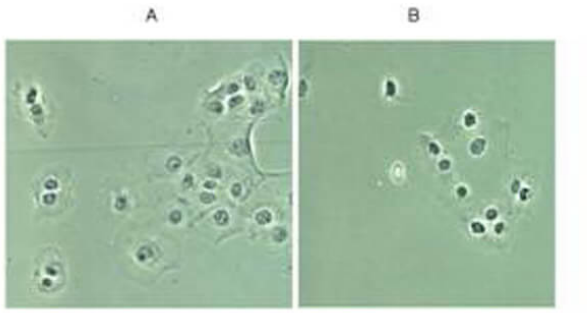
Immunofluorescence Microscopy
NF-κB is activated by stimulation with sera from OSAS patients. (A) A representative picture of a cluster of cells is presented. Cardiomyocytes are identified by staining with anti-cardiac troponin (green). NF-κB subunits: Anti p50 or anti p65 (pink). Arrows point to nuclei expressing p50 or p65. (B) The total average intensity of NF-κB subunits p50 and p65 staining was measured in the nuclei of cardiomyocytes. The cells were incubated each time with 12 different OSAS sera and compared to 10 different control sera (5%). The results presented are an average of 3 separate experiments done in duplicates, showing a significant increase of nuclear p50 and p65 following incubation with OSAS sera. Squares and circles represent individual sera. Statistical significance was determined by the Student's t-test comparing cells incubated with control or OSAS sera (p50 p = 0.01, p65 p = 0.001). * p < 0.05, *** p < 0.01.
Fig 2. PMID: 34768848



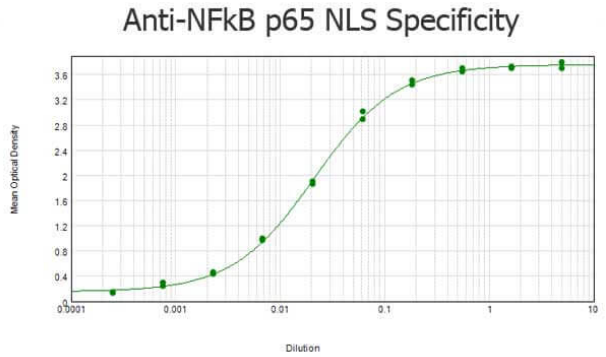
Immunofluorescence Microscopy
Increase in nuclear NF-κB sub-units p65 and p50 expression in hESC-CMs following IH: (A) quantification of the NF-κB subunits p65 and p50 expression in the nucleus following normoxia or IH only on CMs. Normoxia (21% O2, 37 °C), intermittent hypoxia (IH) (1% O2, 37 °C) for 12 h (60 Cycles). Results are averages of 3 separate experiments performed in 10 replicates Student's t-test *** $p < 0.001$, $n = 39,852$; (B) immunostaining of p65, p50 and cardiac troponin T (cardiomyocytes specific marker) following normoxia or IH. Differentiated CMs were detected with antibodies to cardiac troponin T (green) and p65 or p50 (pink), followed by the appropriate secondary antibody. Nuclei were stained with DAPI (blue); (B) normoxia conditions and p65; (C) IH conditions and p65; (D) normoxia conditions and p50; and (E) IH conditions and p50. 40× magnification. Fig 3.
PMID: 36142186



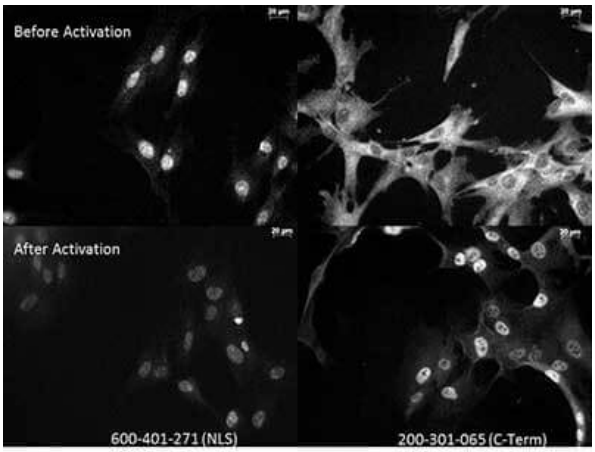
Immunofluorescence Microscopy
Immunofluorescence Microscopy of Anti-p65 NLS Antibody. Cells: TNF stimulated DU145 cells - fixed. Antibody: Rabbit anti-p65 NLS was used at a 1:200 dilution to detect p65. Image shown is at a 1:400 magnification.



Immunofluorescence Microscopy
Rockland Immunochemical's Rabbit anti-p65 NLS was used at a 1:200 dilution to detect p65 in (A) control DU145 cells and (B) TNF stimulated DU145 cells. Although DU145 show relatively high basal levels of nuclear p65 staining, significant enhancement of nuclear staining is seen in panel B as evidence of translocation and availability of the NLS to be bound by the antibody. Cultured cells shown above were formalin-fixed. Tissue staining (not shown) were formalin-fixed, paraffin embedded followed by citrate retrieval. Blocking and hybridization included 5% NGS.



ELISA
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) (p/n 611-103-122) and TMB ELISA Peroxidase Substrate (p/n TMBE-1000).



Immunocytochemistry
Anti-p65 NLS Antibody and Anti NFkB monoclonal antibody – Immunocytochemistry. Tissue: Human Fibroblasts. Top two: Before activation. Bottom two: After activation with poly IC. Left: 600-401-271: anti-p65 NLS specific lot 18372. Right: 200 -301-065: Monoclonal anti-NFkB p65 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.

References

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