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Datasheet for 600-401-265

NFkB p65 phospho S536 Antibody

Overview

Description:	Anti-NFKB p65 (Rel A) pS536 (RABBIT) Antibody - 600-401-265
Item No.:	600-401-265
Size:	100 µg
Applications:	ELISA, IHC, WB, EMSA, IF
Reactivity:	Human
Host Species:	Rabbit

Product Details

Background:	This antibody is designed, produced, and validated as part of a collaboration between Rockland and the National Cancer Institute (NCI) and is suitable for Cancer, Immunology and Nuclear Signaling research. 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), cRel, 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. Lowlevels 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-alpha. IkB-alpha binds to the p65 subunit, preventing nuclear localization, and DNA binding. Activators mediate a rapid phosphorylation of IkB by IkB kinase (IKK), which results in subsequent ubiquitination and proteolytic degradation. NFkB is then transported to the nucleus, where it activates transcription of target genes through binding to NFkB target sequences within the promoter. The HTLV-I protein Tax can induce constitutive NFkB activation through phosphorylation of both IkB-alpha and IkB-beta. The transforming protein Tax inhibits p53 transcriptional activity through the NFkB signaling pathway, specifically via the p65 (RelA) subunit. The inhibition of p53 activity is dependent upon phosphorylation of p65 (RelA) at S536 by the upstream kinase IKKβ.
Synonyms:	rabbit anti-NFKB p65 pS536 antibody, rabbit anti-p65 antibody, rabbit anti-RelA antibody, 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
PTM Specificity:	Phosphorylation
Immunogen Type:	Conjugated Peptide
Immunogen:	This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to a c-terminal region near phospho Serine S536 of human p65 (RelA) protein.
Purity/Specificity:	This product was affinity purified from monospecific antiserum by immunoaffinity chromatography using phospho-peptide coupled to agarose beads followed by solid phase adsorption against nonphospho-peptide. This antibody is specific for human p65 (RelA) protein phosphorylated at S536. A BLAST analysis was used to suggest cross reactivity with p65 (RelA) from human, mouse and rat based on 100% homology with the immunizing sequence. Cross reactivity with p65 (RelA) from other sources has not been determined.
Relevant Links:	• NCBI - 223468676 • UniProtKB - Q04206 • GeneID - 5970

Application Details

Tested Applications:	ELISA, IHC, WB
Suggested Applications:	EMSA, IF (Based on references)
Application Note:	This affinity purified antibody has been tested for use in ELISA, immunohistochemistry and western blotting. Specific conditions for reactivity should be optimized by the end user. By western blot, a band approximately 65 kDa in size corresponding to phosphorylated p65 (RelA) protein is expected in the appropriate cell lysate or extract. This phospho-specific polyclonal antibody reacts with human p65 (RelA) pS536 and shows minimal reactivity by western blot with non-phosphorylated p65 (RelA) and minimal reactivity by ELISA against the non-phosphorylated form of the immunizing peptide.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:1,000 - 1:6,000

IHC:	5 µg/ml
WB:	1:200 - 1:2,000

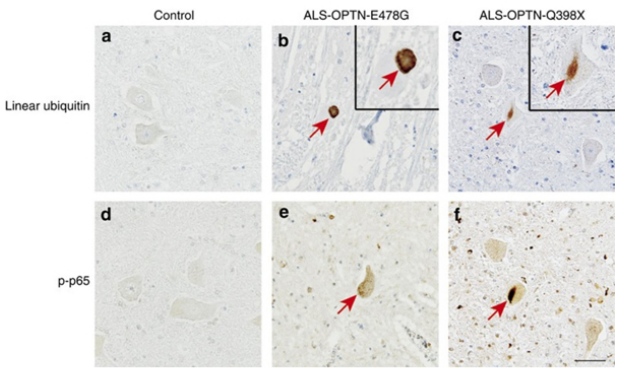
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	1.1 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

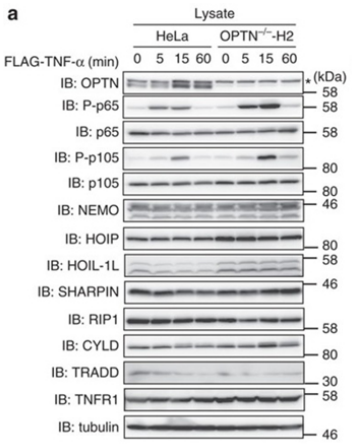
Shipping & Handling

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.

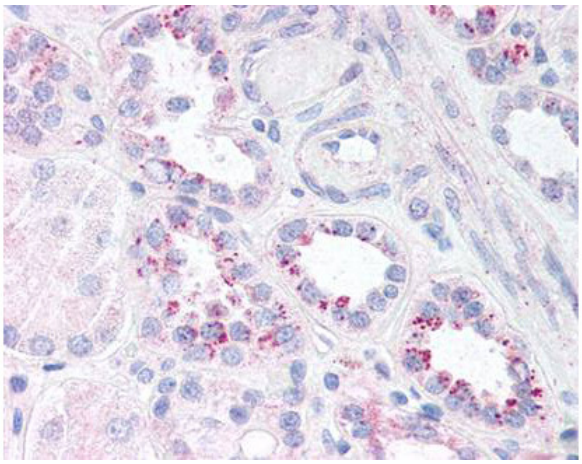
Images



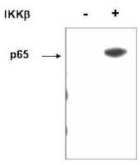
Immunohistochemistry
Immunohistochemical staining of specimens from a leukaemia patient (control) and ALS patients with the heterozygous OPTN-E478G mutation and the homozygous OPTN-Q398X mutation was performed using anti-linear ubiquitin (a–c) and anti-P-p65 (d–f). Arrows indicate immunoreactive inclusions (b,c,e,f) in the spinal anterior horn cells. Scale bar, 50 µm. Fig 6. PMID: 27552911



Western Blot
(a) Parental and OPTN-KO HeLa cells were stimulated with FLAG-TNF-α for the indicated times and then TNFR complex I was immunoprecipitated using an anti-FLAG antibody, followed by immunoblotting using the indicated antibodies. Fig 4. PMID: 27552911



Immunohistochemistry
Rockland's affinity purified anti-p65 (RelA) pS536 antibody was used at 5.0 µg/ml to detect signal in a variety of tissues including multi-human, multi-brain and multi-cancer slides. This image shows moderate positive staining of human kidney distal tubules and collecting ducts. Tissue was formalin-fixed and paraffin embedded. The image shows localization of the antibody as the precipitated red signal, with a hematoxylin purple nuclear counterstain. Personal Communication, Tina Roush, LifeSpanBiosciences, Seattle, WA.



Western Blot
Western blot using Rockland's affinity purified anti-p65 (RelA) pS536 antibody shows detection of p65 phosphorylated at S536. The control blot (left lane) contains 100 ng of purified p65-GST fusion protein. The band is seen (right lane) when this protein is phosphorylated at S536 by IKKß. Personal Communication. J. Brady, NCI, Bethesda, MD.

References

- Nakayama Y et al. Linear polyubiquitin chain modification of TDP-43-positive neuronal cytoplasmic inclusions in amyotrophic lateral sclerosis. *J Neuropathol.* (2020)
- Yamaguchi Y et al. Phosphorylated NF-κB subunit p65 aggregates in granulovacuolar degeneration and neurites in neurodegenerative diseases with tauopathy. *Neurosci Lett.* (2019)
- Nakazawa et al. Linear ubiquitination is involved in the pathogenesis of optineurin-associated amyotrophic lateral sclerosis. *Nature Communications* (2016)
- Ryu, S et al. Suppression of Propionibacterium acnes Infection and the Associated Inflammatory Response by the Antimicrobial Peptide P5 in Mice. *PloS One* (2015)
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