

**Certificate of Analysis**
**Product:** Affinity Purified Anti-NF $\kappa$ B p65 (RelA) pS536 (Rabbit)

**Code:** 600-401-265

**Lot #:** 18835

**Size:** 100  $\mu$ g

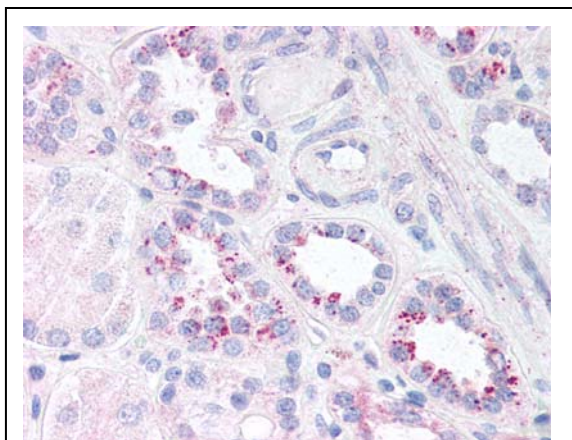
**Physical State:** Liquid (sterile filtered)

**Antibody Concentration:** 0.9 mg/ml (by UV absorbance at 280 nm)

**Buffer:** 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

**Stabilizer:** None

**Preservative:** 0.01% (w/v) Sodium Azide

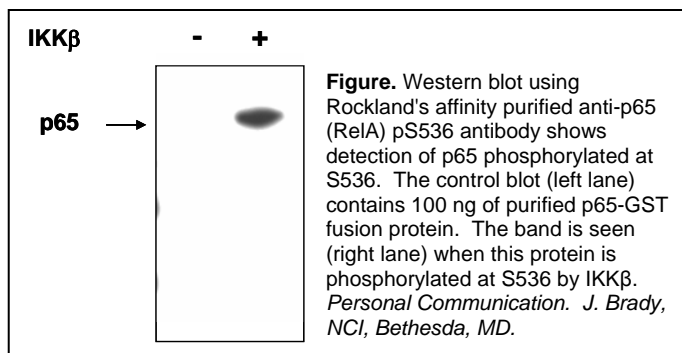
**Storage Conditions:** Store vial at  $-20^{\circ}$  C or below prior to opening. Dilute only prior to immediate use. For extended storage, aliquot contents and freeze at  $-20^{\circ}$  C or below. Avoid cycles of freezing and thawing. Expiration date is one (1) year from date of opening.


**Figure 2.** Immunohistochemistry. ROCKLAND's affinity purified anti-p65 (RelA) pS536 antibody was used at 5.0  $\mu$ g/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.*

**Background Information:** NF $\kappa$ B 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 NF $\kappa$ B bound to I $\kappa$ B. NF $\kappa$ B was originally identified as a heterodimeric DNA binding protein complex consisting of p65 (RelA) and p50 (NF $\kappa$ B1) subunits. Other identified subunits include p52 (NF $\kappa$ B2), 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 NF $\kappa$ B 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 I $\kappa$ B- $\alpha$ . I $\kappa$ B- $\alpha$  binds to the p65 subunit, preventing nuclear localization, and DNA binding. Activators mediate a rapid phosphorylation of I $\kappa$ B by I $\kappa$ B kinase (IKK), which results in subsequent ubiquitination and proteolytic degradation. NF $\kappa$ B is then transported to the nucleus, where it activates transcription of target genes through binding to NF $\kappa$ B target sequences within the promoter. The HTLV-I protein Tax can induce constitutive NF $\kappa$ B activation through phosphorylation of both I $\kappa$ B- $\alpha$  and I $\kappa$ B- $\beta$ . The transforming protein Tax inhibits p53 transcriptional activity through the NF $\kappa$ B 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 IKK $\beta$ .

**Recommended Dilutions:**

<b>ELISA</b>	1:1,000 - 1:6,000
<b>WESTERN BLOT</b>	1:200 - 1:2,000
<b>IMMUNOHISTOCHEMISTRY</b>	5 $\mu$ g/ml
<b>OTHER APPLICATIONS</b>	User Optimized



**Application Note(s):** 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.

**Purity and 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.

**Immunogen:** This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to residues surrounding S536 of human p65 (RelA) protein.

**Relevant Links:** NCBI [Q04206](#) Swiss-Prot [Q04206](#)

#### Related Products:

<a href="#">#100-4165</a>	Anti-NFKB p65 (Rel A) (RABBIT)
<a href="#">#100-401-264</a>	Anti-NFKB p65 (Rel A) pS276 (Rabbit)
<a href="#">#100-401-266</a>	Anti-NFKB p65 (Rel A) pS529 (Rabbit)
<a href="#">#600-401-271</a>	Anti-NFKB p65 NLS specific (Rabbit)
<a href="#">#100-4165N</a>	Anti-NFKB p65 (Rel A) N-TERMINAL SPECIFIC (Rabbit)
<a href="#">#611-703-127</a>	Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (DONKEY) MX10
<a href="#">#611-132-122</a>	IRDye® 800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
<a href="#">#B501-0500</a>	BLOTTO (500 g)
<a href="#">#BSA-30</a>	30% BOVINE SERUM ALBUMIN SOL'N in 0.85% sodium chloride (no preservative or stabilizer) (500 ml)
<a href="#">#B304</a>	NORMAL GOAT SERUM (NGS) (10 ml)
<a href="#">#KIA-003</a>	<b>MaxTag™</b> Anti-RABBIT IgG Kit for Immunoblotting
<a href="#">#MB-070</a>	Blocking Buffer for Fluorescent Western Blotting

#### General References:

Jeong S.J., Pise-Masison C.A., Radonovich M.F., Park H.U., and Brady J.N. (2005) A Novel NF- $\kappa$ B Pathway Involving IKK $\beta$  and p65/RelA Ser-536 Phosphorylation Results in p53 Inhibition in the Absence of NF $\kappa$ B Transcriptional Activity. *J. Biol. Chem.* **280** (11): 10326-10332.

Baldwin, A.S. (2001) Control of oncogenesis and cancer therapy resistance by the transcription factor NF $\kappa$ B. *J. Clinical Investigation* **107**: 241-246.

Baldwin, A.S. (2001) Series introduction: the transcription factor NF $\kappa$ B and human disease. *J. Clinical Investigation* **107**: 3-6.

**Note:** This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information.

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