

**Datasheet for 100-401-264****NFkB p65 (RelA) Phospho S276 Antibody****Overview**

|                      |                                                             |
|----------------------|-------------------------------------------------------------|
| <b>Description:</b>  | Anti-NFkB p65 (Rel A) pS276 (RABBIT) Antibody - 100-401-264 |
| <b>Item No.:</b>     | 100-401-264                                                 |
| <b>Size:</b>         | 100 µL                                                      |
| <b>Applications:</b> | ELISA, IHC, WB, IF                                          |
| <b>Reactivity:</b>   | Human                                                       |
| <b>Host Species:</b> | Rabbit                                                      |

**Product Details****Background:**

NF-κB 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-κB bound to IκB. NF-κB was originally identified as a heterodimeric DNA binding protein complex consisting of p65 (RelA) and p50 (NFκB1) subunits. Other identified subunits include p52 (NFκB2), 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-κB 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 IκB-α. IκB-α binds to the p65 subunit preventing nuclear localization and DNA binding. Activators mediate a rapid phosphorylation of IκB by IκB kinase (IKK) which results in subsequent ubiquitination and proteolytic degradation. NF-κB is then transported to the nucleus, where it activates transcription of target genes through binding to NF-κB target sequences within the promoter. The HTLV-I protein Tax can induce constitutive NF-κB activation through phosphorylation of both IκB-α and IκB-β. The transforming protein Tax inhibits p53 transcriptional activity through the NFκB 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. Anti-NFκB antibody is ideal for Cell Biology, Nuclear Signaling, Neuroscience and Signal Transduction Research.

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|----------------------|-----------------------------------------------------------------------------------------------------------------------------------|
| <b>Synonyms:</b>     | rabbit anti-NFkB p65 pS276 Antibody, rabbit anti-RelA pS276 Antibody, NFkB, nfkb, NF-κB, NF-kappaB, NFkappaB, Anti-NF-κB antibody |
| <b>Host Species:</b> | Rabbit                                                                                                                            |
| <b>Clonality:</b>    | Polyclonal                                                                                                                        |

**Format:** Antiserum

## Target Details

|                            |                                                                                                                                                                                                                                                                                                                                                                              |
|----------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Gene Name:</b>          | RELA                                                                                                                                                                                                                                                                                                                                                                         |
| <b>Reactivity:</b>         | Human                                                                                                                                                                                                                                                                                                                                                                        |
| <b>PTM Specificity:</b>    | Phosphorylation                                                                                                                                                                                                                                                                                                                                                              |
| <b>Immunogen Type:</b>     | Conjugated Peptide                                                                                                                                                                                                                                                                                                                                                           |
| <b>Immunogen:</b>          | NFkB p65 (Rel A) peptide corresponding to an internal region near phospho Serine 276 of the human protein conjugated to Keyhole Limpet Hemocyanin (KLH).                                                                                                                                                                                                                     |
| <b>Purity/Specificity:</b> | Anti-NFkB p65 (RelA) Phospho S276 Antibody was prepared from monospecific antiserum by delipidation and defibrination. This phospho specific polyclonal antibody is specific for phosphorylated pS276 human p65. Reactivity with non-phosphorylated p65 is minimal. Cross reactivity with pS276 phosphorylated p65 from mouse, rat or other species has not been determined. |
| <b>Relevant Links:</b>     | <ul style="list-style-type: none"><li>• <a href="#">UniProtKB - Q04206</a></li><li>• <a href="#">NCBI - 223468676</a></li><li>• <a href="#">GenelD - 5970</a></li></ul>                                                                                                                                                                                                      |

## Application Details

|                                |                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |
|--------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Tested Applications:</b>    | ELISA, IHC, WB                                                                                                                                                                                                                                                                                                                                                                                                                                                              |
| <b>Suggested Applications:</b> | IF (Based on references)                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <b>Application Note:</b>       | Phospho NFkB antibody reacts human pS276 p65 and shows minimal reactivity by western blot with non-phosphorylated p65 and minimal reactivity by ELISA against the non-phosphorylated form of the immunizing peptide. A 1:500 dilution has been used for staining p65 in human kidney tissue by IHC. Tissue was formalin fixed and paraffin embedded. Although not tested, this antibody is likely functional in immunoprecipitation. All conditions must be user optimized. |
| <b>Assay Dilutions:</b>        | All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.                                                                                                                                                                                                                                                                                                                                                                             |
| <b>ELISA:</b>                  | 1:10,000 - 1:30,000                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>IHC:</b>                    | 1:200-1:800                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>WB:</b>                     | 1:1,000 - 1:5,000                                                                                                                                                                                                                                                                                                                                                                                                                                                           |

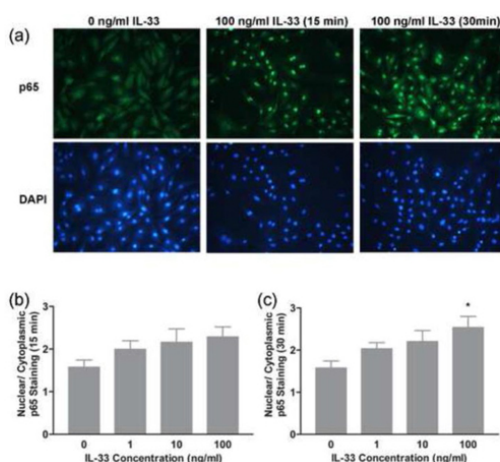
## Formulation

|                 |                           |
|-----------------|---------------------------|
| Physical State: | Liquid (sterile filtered) |
| Concentration:  | 80 mg/mL by Refractometry |
| Buffer:         | None                      |
| Preservative:   | 0.01% (w/v) Sodium Azide  |
| Stabilizer:     | None                      |

## Shipping & Handling

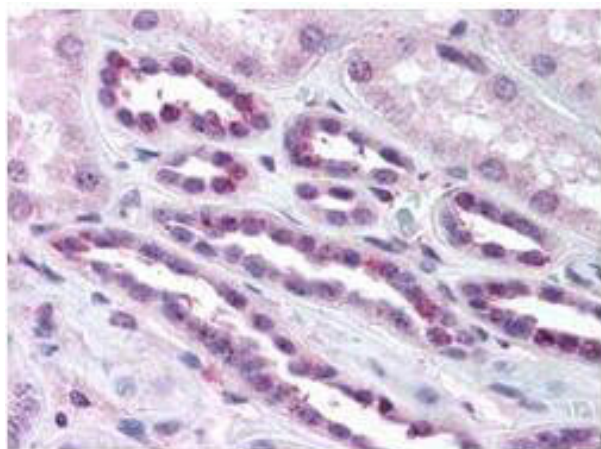
|                     |                                                                                                                                                                                                                                                                                                                                               |
|---------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Shipping Condition: | Dry Ice                                                                                                                                                                                                                                                                                                                                       |
| Storage Condition:  | Store vial at -20° C 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



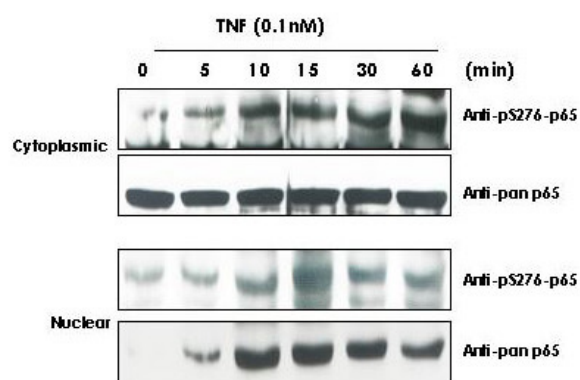
### Immunocytochemistry

Immunocytochemical Staining using NF-kB p65. For examination of NF-kB activation, following 15 to 30 minutes of IL-33 treatment, rat cardiac fibroblasts were fixed and stained immunocytochemically with an NF-kB antibody to evaluate the translocation of NF-kB. Cells demonstrated significant NF-kB activation indicated by enhanced translocation of NF-kB from the cytoplasm to the nucleus of the cells after 30 minutes of IL-33 treatment. This was a dose-dependent response (Fig. 6a–c). Thus, we demonstrated that IL-33 treatment can rapidly activate multiple MAPKs as well as the NF-kB pathway in adult rat cardiac fibroblasts. Fig 6. PMID: 22445500



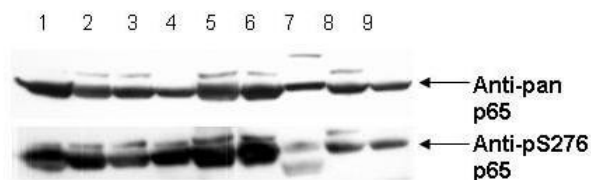
### Immunohistochemistry

Rockland's anti-p65 pS276 antibody was diluted 1:500 to detect p65 in human kidney tissue. Tissue was formalin fixed and paraffin embedded. No pre-treatment of sample was required. The image shows the localization of antibody as the precipitated red signal, with a hematoxylin purple nuclear counter stain.



### Western Blot

TNF Induces phosphorylation of p65 in KBM-5 cells. Cytoplasmic and nuclear protein lysates prepared after 0, 5, 10, 15, 30 and 60 minutes of 0.1 nM TNF treatment of KBM-5 cells shows inducible phosphorylation using phospho specific polyclonal anti-human pS276 p65. Rockland Immunochemical's pan reactive anti p65 (code# 100-4165) was used as a control to show the presence of total p65 in both the cytoplasmic and nuclear extracts. Phosphorylation of p65 occurs after approximately 10 min of TNF exposure. Migration of phosphorylated p65 into the nucleus occurs within a similar time frame. HRP conjugated Gt-anti-Rabbit IgG was used to develop the blot using a chemi-luminescent detection method. Other detection methods will yield similar results. Personal Communication, Aggarwal BB



### Western Blot

Anti-pS276 shows phospho p65 staining in carcinoma cells. Western blot of total protein lysates from various human head and neck tumors shows phospho p65 staining in tumor cell lines using phospho specific polyclonal anti-human pS276 p65. Lanes 1-6 contain protein lysates from human squamal carcinoma cell lines. Lane 7 is a protein lysate from a primary culture of human keratinocytes and does not show significant levels of phosphorylated p65. Lane 8 contains protein lysate from ATCC SCC9 cells (also a head and neck squamal carcinoma). Lane 9 contains lysate from EGF-induced human derived A431 cells. Lane 10 (not shown) contains a molecular weight standard. Concurrent staining with anti-beta microtubulin (not shown) was used to confirm equal protein loading in all lanes. HRP conjugated Gt-anti-Rabbit IgG was used to develop the blot using a chemiluminescent detection method. Other detection methods will yield similar results. Data contributed by Yu, M., NIH, personal communication.

## References

- Lai et al. MicroRNA-33 Regulates the Innate Immune Response via ATP Binding Cassette Transporter-mediated Remodeling of Membrane Microdomains. *Journal of Biological Chemistry* (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)
- Zhu J. et al. Effects of interleukin-33 on cardiac fibroblast gene expression and activity. *Cytokine* (2012)
- Spooren A, et al. Hunting for serine 276-phosphorylated p65. *J Biomed Biotechnol.* (2010)
- Begley et al. CXCL5 promotes prostate cancer progression. *Neoplasia* (2008)
- Begley et al. CXCL12 overexpression and secretion by aging fibroblasts enhance human prostate epithelial proliferation in vitro. *Aging Cell.* (2005)
- Chapman et al. Expression and deoxyribonucleic acid-binding activity of the nuclear factor kappaB family in the human myometrium during pregnancy and labor. *J Clin Endocrinol Metab.* (2004)

## Disclaimer

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. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.