

**Datasheet for 200-301-065****NFkB p65 Antibody****Overview**

|                      |                                                                 |
|----------------------|-----------------------------------------------------------------|
| <b>Description:</b>  | Anti-NFKB p65 (Rel A) (MOUSE) Monoclonal Antibody - 200-301-065 |
| <b>Item No.:</b>     | 200-301-065                                                     |
| <b>Size:</b>         | 100 µg                                                          |
| <b>Applications:</b> | IF, IHC, WB, IP                                                 |
| <b>Reactivity:</b>   | Human                                                           |
| <b>Host Species:</b> | Mouse                                                           |

**Product Details**

|                      |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |
|----------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Background:</b>   | NFkappaB was originally identified as a factor that binds to the immunoglobulin kappa light chain enhancer in B cells. 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. Cell Biology, Nuclear Signaling, Neuroscience and Signal Transduction Research. |
| <b>Synonyms:</b>     | mouse anti-NF-kB p65 Antibody, mouse anti-Rel A antibody, NFKB, nfkb, NF-kB, NF-kappaB, NFkappaB, Nuclear factor NF-kappa-B p65 subunit                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>Host Species:</b> | Mouse                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
| <b>Clonality:</b>    | Monoclonal                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |
| <b>Clone ID:</b>     | 27F9.G4                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |
| <b>Format:</b>       | IgG2a                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |

**Target Details**

|                        |                    |
|------------------------|--------------------|
| <b>Gene Name:</b>      | RELA               |
| <b>Reactivity:</b>     | Human              |
| <b>Immunogen Type:</b> | Conjugated Peptide |

|                            |                                                                                                                                                                                                                                                                                                                                            |
|----------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Immunogen:</b>          | NFkB p65 (Rel A) peptide corresponding to a region near the C-terminus of the human protein conjugated to Keyhole Limpet Hemocyanin (KLH).                                                                                                                                                                                                 |
| <b>Purity/Specificity:</b> | Anti-NF-kB p65 Antibody was purified from concentrated tissue culture supernate by Protein A chromatography and showed a single band by IEP (immunoelectrophoresis) when tested with anti-mouse antibody. Reactivity was confirmed by ELISA against peptide conjugated carrier protein and by Western blot against HeLa whole cell lysate. |
| <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="#">GeneID - 5970</a></li></ul>                                                                                                                                                                    |

## Application Details

|                                |                                                                                                                                                                                                                                                                   |
|--------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Tested Applications:</b>    | IF, IHC, WB                                                                                                                                                                                                                                                       |
| <b>Suggested Applications:</b> | IP (Based on references)                                                                                                                                                                                                                                          |
| <b>Application Note:</b>       | Anti-NF-kB p65 Antibody is a mouse monoclonal antibody directed against NFkB p65 (Rel A) and recognizes a 65 kD band by Western blot against HeLa whole cell lysate. Control peptide (100-4165p) is sold separately. This product tested in WB, ICC, IHC, and IF. |
| <b>Assay Dilutions:</b>        | All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.                                                                                                                                                                   |
| <b>ELISA:</b>                  | 1:50,000-1:100,000                                                                                                                                                                                                                                                |
| <b>IF:</b>                     | 1:5,000                                                                                                                                                                                                                                                           |
| <b>IHC:</b>                    | 1:200-1:600                                                                                                                                                                                                                                                       |
| <b>WB:</b>                     | 1:1,000-1:5,000                                                                                                                                                                                                                                                   |

## Formulation

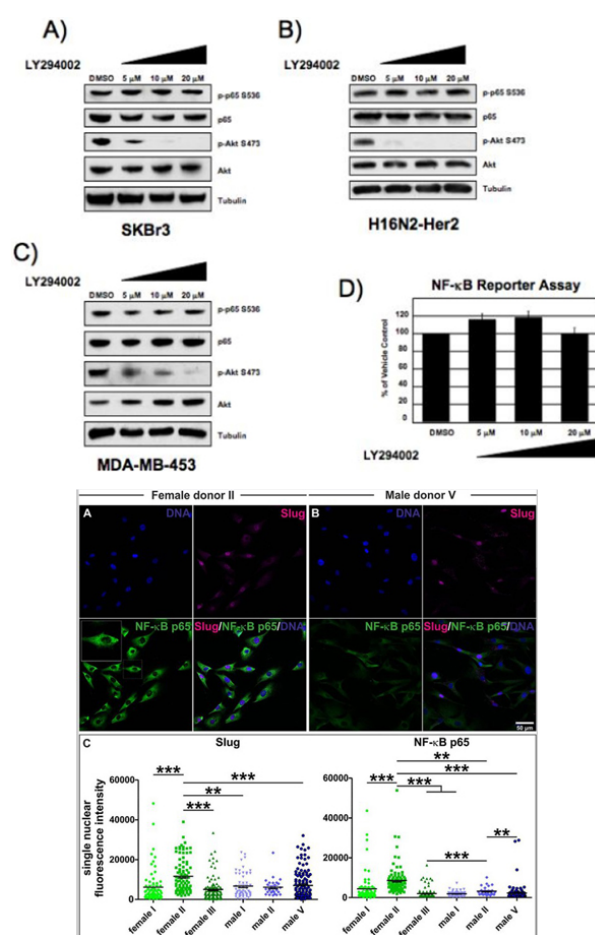
|                        |                                                            |
|------------------------|------------------------------------------------------------|
| <b>Physical State:</b> | Liquid (sterile filtered)                                  |
| <b>Concentration:</b>  | 0.98 mg/mL by UV absorbance at 280 nm                      |
| <b>Buffer:</b>         | 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2 |
| <b>Preservative:</b>   | 0.01% (w/v) Sodium Azide                                   |
| <b>Stabilizer:</b>     | None                                                       |

## Shipping & Handling

|                            |         |
|----------------------------|---------|
| <b>Shipping Condition:</b> | Dry Ice |
|----------------------------|---------|

|                           |                                                                                                                                                                                                                                                                                                                                                                                                                   |
|---------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Storage Condition:</b> | Store NF- $\kappa$ B p65 Antibody at $-20^{\circ}\text{C}$ prior to opening. Aliquot contents and freeze at $-20^{\circ}\text{C}$ 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 $4^{\circ}\text{C}$ as an undiluted liquid. Dilute only prior to immediate use. |
| <b>Expiration:</b>        | Expiration date is one (1) year from date of receipt.                                                                                                                                                                                                                                                                                                                                                             |

## Images



### Western Blot

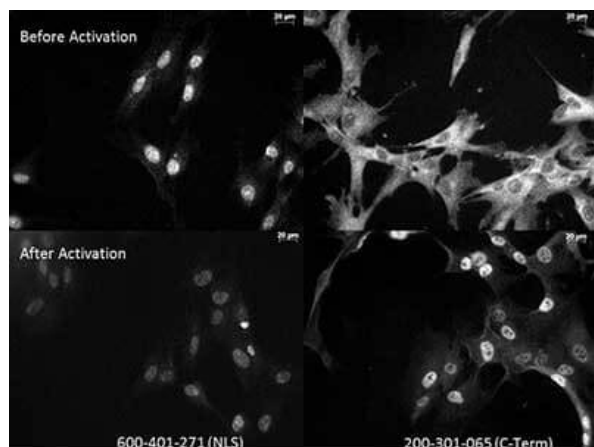
Western blot of phospho-p65 serine 536 from SKBr3 (A), H16N2-Her2 (B) and MDA-MB-453 (C) cells treated with PI3K-inhibitor LY294002 for 2 hours. Western blot analysis was performed with 25  $\mu\text{g}$  whole cell extracts. D) Luciferase reporter assay of SKBr3 cells treated with LY294002 overnight. Fold change of reporter activity with PI3K-inhibitor treatment is shown relative to vehicle treated cells. Values are the average of at least 3 experiments. Error bars are  $\pm 1$  S.E. Samples are normalized by protein concentration.

Figure 5. PMID: 19946332

### Immunofluorescence Microscopy

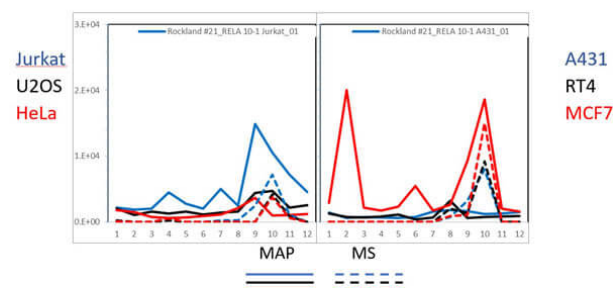
Intra- and interpopulational heterogeneity of NF- $\kappa$ B-p65 and the EMT transcription factor Slug. A-B: Representative images of immunocytochemical stainings showing heterogeneity of Slug and NF- $\kappa$ B-p65 protein between single ITSCs and ITSC-populations. C: Quantification of immunocytochemical stainings via measurement of the nuclear fluorescence intensity of Slug and NF- $\kappa$ B-p65 per single cell revealed an intrapopulational and interpopulational variability with regard to the donor. Kruskal-Wallis test, Post test: Dunn's Multiple Comparison Test, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ . was considered significant.

Fig 3. PMID: 34748196



### Immunocytochemistry

Anti NFkB monoclonal antibody – Immunocytochemistry.  
Tissue: Human Fibroblasts. Top: Before activation. Bottom: After activation with poly IC. Left: Anti-p65 NLS specific - (p/n 600-401-271, lot 18372). Right: Anti-p65 C-Term monoclonal antibody - (p/n 200-301-065, lot 26076). 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.

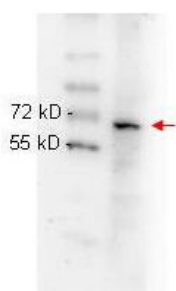


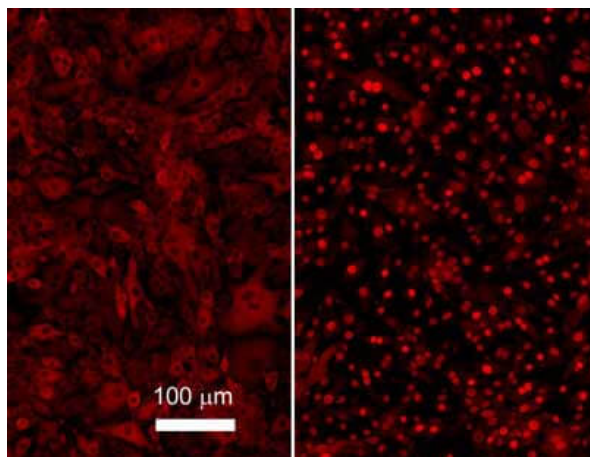
### PAGE-MAP

PAGE-MAP (microsphere affinity proteomics) of Mouse Anti-NFkB p65 (Rel A) Antibody. (Catalog Number: 200-301-065, Lot Number: 26076). Antibody array western blot binding of gelfree size separated fractions of multiple lysates (solid lines) and shotgun mass spectroscopy identification (dashed lines) of the target band run in parallel correlate confirming the specificity of this antibody against NFkB p65. Data was provided by the Lund-Johansen lab of Oslo University Hospital. For more information on PAGE-MAP/IP-MS identification of antibody specificity and its large-scale implementation for antibody validation see Sikorski et. al., (2018) Nature Methods 15, 909-912.

### Western Blot

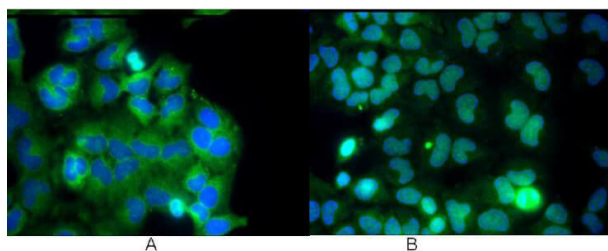
Rockland anti NFkB p65 (Rel A) monoclonal antibody (p/n 200-301-065) was used to detect ~65 kD band (red arrow) in HeLa whole cell lysate (p/n W09-000-364). Lysate was run on 4-20% gradient gel transferred under standard conditions and blocked in 1% BSA-TBST for 30 min at RT. Blot was probed with monoclonal anti-NFkB p65 at 1:1000 in 1% BSA-TBST o/n at 4°C and detected with HRP conjugated Rb-anti-Mouse antibody (p/n 610-4302) at 1:40,000 in (p/n MB-070) for 30 min at RT.





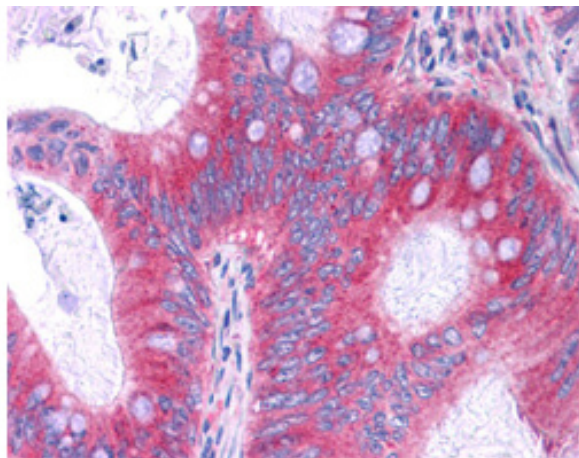
### Immunofluorescence Microscopy

Rockland Monoclonal anti NFkB p65 (Rel A) antibody was used to detect p65 in b.end5 mouse endothelial cells. Unstimulated control cells (left) show cytoplasmic staining, TNF-alpha stimulated cells (right) show nuclear staining. For staining, cells were washed with PBS to remove all traces of culture media and fixed with paraformaldehyde 4 % (45 min). Slides were washed with PBT (phosphate buffer 0.1M + Triton-X-100 0.1 %), 3 times 10 min each, then, blocked with PBT + 5 % normal goat serum, 1 hour. Sample was incubated overnight in primary antibody (1:600 in blocking buffer). After 3X wash in PBT for 10 min, slides were incubated 1 hour with secondary antibody (1:1000) and mounted in 1:1 PB glycerol. Images kindly provided by Tebu-Bio from Francisco Javier Carrillo-Salinas (PhD Student); Dra. Carmen Guaza Instituto Cajal (CSIC), Madrid, Spain.



### Immunofluorescence Microscopy

Rockland Monoclonal anti NFkB p65 (Rel A) antibody was used to detect p65 by immunofluorescence at a dilution of 1:5000. HeLa cells were grown to sub-confluent on 18 mm<sup>2</sup> glass coverslips #1.5. Cells were either unstimulated (A), or stimulated (B) with 50 ng/ml of TNF alpha for 30 min prior fixation. Cells were then fixed in methanol and blocked with 10% normal goat serum (NGS), in PBS, and TritonX 0.2% (Tx) and incubated for 1 hr at RT with primary ab, counterstained with DAPI and washed in PBS/NGS/Tx. Cells were incubated for 1 hr at RT with Atto 425 conjugated anti mouse secondary antibody for STED CW imaging. Data was collected on a STED-CW TCS-SP5 Confocal system equipped with a DFC 350FX camera allowing sequential acquisition in widefield, confocal and STED CW imaging on the same system.



### Immunohistochemistry

Rockland Antibody (p/n 200-301-065) has been tested in immunohistochemistry, analyzed by an anatomic pathologist and validated for use in IHC applications against formalin-fixed, paraffin-embedded human tissues. Showed moderate to strong staining within many tissues, including epithelium of the breast, small intestine, kidney, pancreas, prostate, skin, placenta, and uterus, as well as within neurons and lymphoid tissues such as spleen, thymus, and tonsil. The antibody produced an excellent signal with almost no background staining at a concentration of 2.5µg/ml. The image displayed shows specific staining in colon carcinoma as the precipitated red signal, with a hematoxylin purple nuclear counterstain. Image provided courtesy of LifeSpan Biosciences, Seattle, WA.

## References

- Yokoi H et al. Erythritol Can Inhibit the Expression of Senescence Molecules in Mouse Gingival Tissues and Human Gingival Fibroblasts. *Nutrients*. (2023)
- Windmöller, BA et al. Inter- and Intrapopulational Heterogeneity of Characteristic Markers in Adult Human Neural Crest-derived Stem Cells. *Stem Cell Reviews and Reports* (2022)
- Chansard A et al. Unveiling Interindividual Variability of Human Fibroblast Innate Immune Response Using Robust Cell-Based Protocols. *Front Immunol*. (2021)
- Sikorski et al. A high-throughput pipeline for validation of antibodies. *Nature Methods* (2018)
- Ryu, S et al. Suppression of *Propionibacterium acnes* Infection and the Associated Inflammatory Response by the Antimicrobial Peptide P5 in Mice. *PloS One* (2015)
- Merkhofer EC et al. Her2 Activates NF-κB and Induces Invasion Through the Canonical Pathway Involving IKKα. *Oncogene*. (2010)

## Disclaimer

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