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Datasheet for 200-301-065S

NFkB p65 Antibody

Overview

Description:	Anti-NFKB p65 (Rel A) (MOUSE) Monoclonal Antibody - 200-301-065S
Item No.:	200-301-065S
Size:	25 µL
Applications:	IF, IHC, WB, IP
Reactivity:	Human
Host Species:	Mouse

Product Details

Background:	NFkappaB 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 NFkappaB bound to IkappaB. NFkappaB 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 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. IkappaBalpha-□□binds to the p65 subunit, preventing nuclear localization and DNA binding. Low levels of p52 and p50 homodimers can also exist in cells.
Synonyms:	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
Host Species:	Mouse
Clonality:	Monoclonal
Clone ID:	27F9.G4
Format:	IgG2a

Target Details

Gene Name:	RELA

Reactivity:	Human
Immunogen Type:	Conjugated Peptide
Immunogen:	NFkB p65 (Rel A) peptide corresponding to a region near the C-terminus of the human protein conjugated to Keyhole Limpet Hemocyanin (KLH).
Purity/Specificity:	This product 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.
Relevant Links:	• NCBI - 223468676 • UniProtKB - Q04206 • GeneID - 5970

Application Details

Tested Applications:	IF, IHC, WB
Suggested Applications:	IP (Based on references)
Application Note:	This protein A purified mouse monoclonal antibody is directed against NFkB p65 (Rel A) and a 1:1000 dilution 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.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:50,000-1:100,000
IF:	1:5000
IHC:	1:200-1:600
WB:	1:1000 - 1:5000

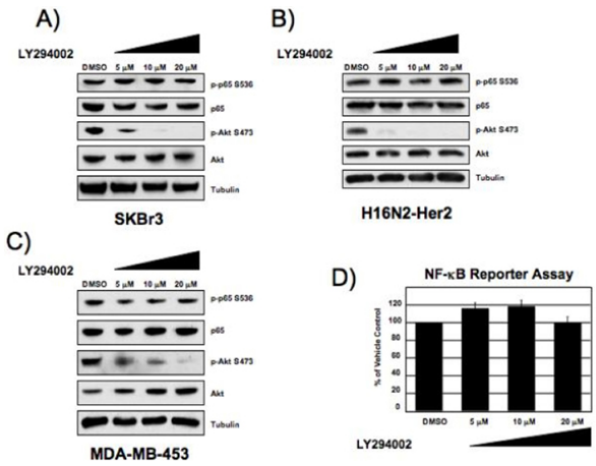
Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	0.98 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

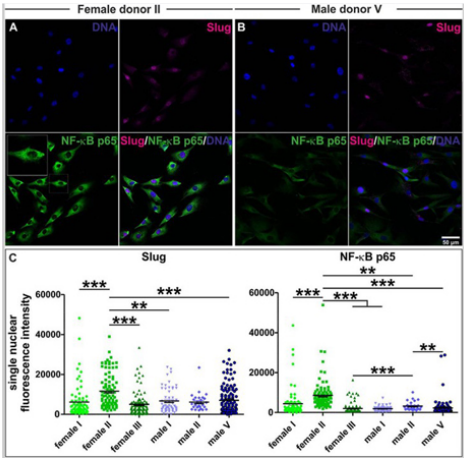
Shipping & Handling

Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at -20°C or below after dilution. Avoid cycles of freezing and thawing.
Expiration:	Expiration date is three (3) months from date of receipt.

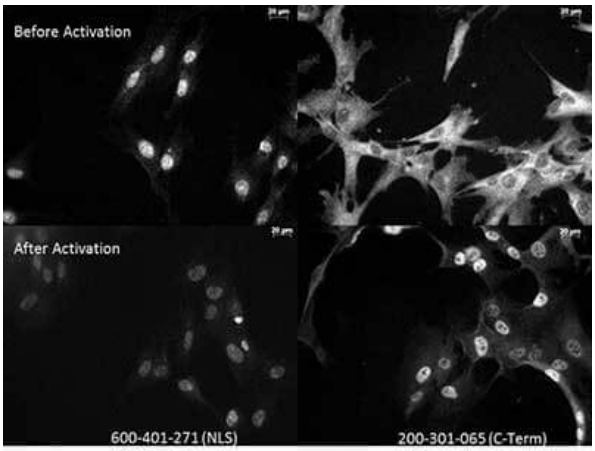
Images



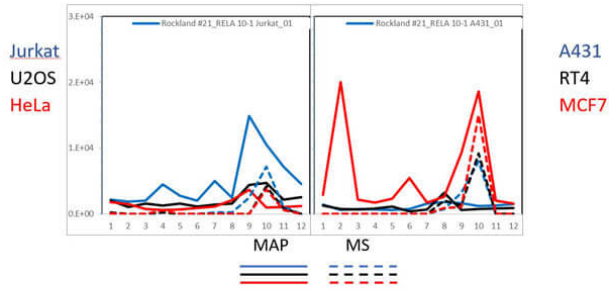
Western Blot
Western blot of phospho-p65 serine 536 from SKBr3 (A), H16N2-Her2 (B) and MDA-MB-453 (C) cells treated with PI3K-inhibitor inhibitor LY294002 for 2 hours. Western blot analysis was performed with 25 µg whole cell extracts. D) Luciferase reporter assay of SKBr3 cells treated with LY294002 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 ± 1 S.E. Samples are normalized by protein concentration.
Figure 5. PMID: 19946332



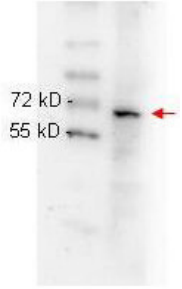
Immunofluorescence Microscopy
Intra- and interpopulational heterogeneity of NF-κB-p65 and the EMT transcription factor Slug. A-B: Representative images of immunocytochemical stainings showing heterogeneity of Slug and NF-κB-p65 protein between single ITSCs and ITSC-populations. C: Quantification of immunocytochemical stainings via measurement of the nuclear fluorescence intensity of Slug and NF-κB-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



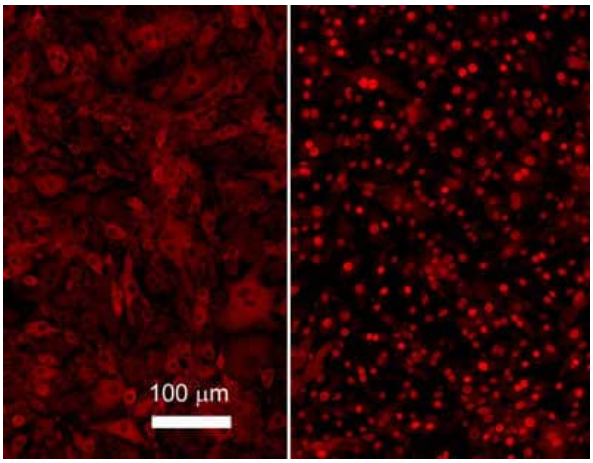
Immunocytochemistry
Anti NFkB monoclonal antibody – Immunocytochemistry. Tissue: Human Fibroblasts. Top: Before activation. Bottom: After activation with poly IC. Left: Anti-p65 NLS specific - (p/n 600-401-271, lot 18372). Right: Anti-p65 C-Term monoclonal antibody - (p/n 200-301-065, lot 26076). The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



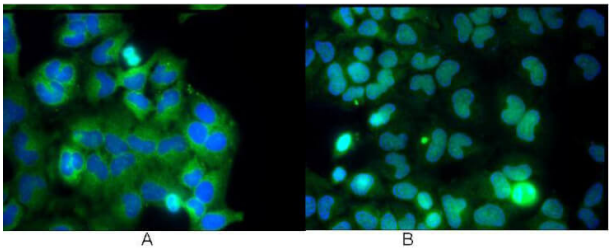
PAGE-MAP
PAGE-MAP (microsphere affinity proteomics) of Mouse Anti-NFKB p65 (Rel A) Antibody. (Catalog Number: 200-301-065, Lot Number: 26076). Antibody array western blot binding of gelfree size separated fractions of multiple lysates (solid lines) and shotgun mass spectroscopy identification (dashed 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.



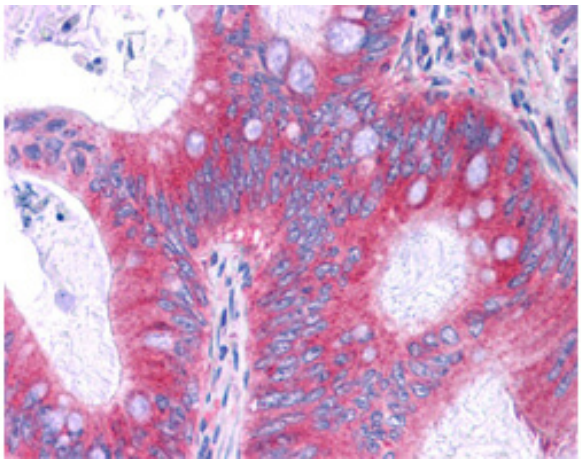
Western Blot
Rockland anti NFKB p65 (Rel A) monoclonal antibody (p/n 200-301-065) was used to detect ~65 kD band (red arrow) in HeLa whole cell lysate (p/n W09-000-364). Lysate was run on 4-20% gradient gel transferred under standard conditions and blocked in 1% BSA-TBST for 30 min at RT. Blot was probed 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 mm2 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 widefiled, 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

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