Product Datasheet ReIA/NFkB p65 Antibody (27F9.G4) NBP1-77815

Unit Size: 0.1 mg

Store at -20C short term. Aliquot and store at -80C long term. Avoid freeze-thaw cycles.

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NBP1-77815

ReIA/NFkB p65 Antibody (27F9.G4)

Product Information	
Unit Size	0.1 mg
Concentration	Please see the vial label for concentration. If unlisted please contact technical services.
Storage	Store at -20C short term. Aliquot and store at -80C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	27F9.G4
Preservative	0.01% Sodium Azide
Isotype	IgG2a Kappa
Purity	Protein A purified
Buffer	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Product Description	
Description	 This 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 Store this antibody at -20C prior to opening. Aliquot contents and freeze at -20C 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 4C as an undiluted liquid. Dilute only prior to
11	immediate use.
Host	Mouse
Gene ID	5970
Gene Symbol	RELA
Species	Human
Specificity/Sensitivity	Reactivity was confirmed by ELISA against peptide conjugated carrier protein and by Western blot against HeLa whole cell lysate.
Immunogen	RelA/NFkB p65 peptide corresponding to a region near the C-terminus of the human protein conjugated to Keyhole Limpet Hemocyanin (KLH). (Uniprot: Q04206)
Product Application Details	
Applications	Western Blot, Dot Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin
Recommended Dilutions	Western Blot 1:1000-1:5000, Immunohistochemistry 1:200-1:600, Immunocytochemistry/ Immunofluorescence 1:5000, Immunohistochemistry- Paraffin 1:10-1:500, Dot Blot
Application Notes	This product 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.



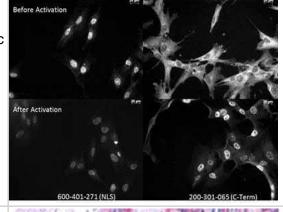
Images

Western Blot: RelA/NFkB p65 Antibody (27F9.G4) [NBP1-77815] -RelA/RelA/NFkB p65 p65 antibody (27F9.G4) was used to detect p65 by Western blot. Samples were prepared in RIPA lysis buffer, boiled with NuPage 4x LDS Sample Buffer and run on NuPage 4-12% Bis-Tris Gels. Blot was incubated with primary antibody at a dilution of 1:500 and detected with HRP conjugated anti-mouse antibody at a dilution of 1:10000.

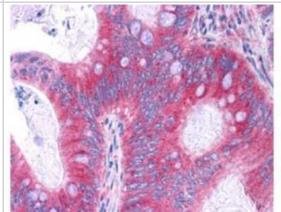
Immunocytochemistry/Immunofluorescence: ReIA/NFkB p65 Antibody (27F9.G4) [NBP1-77815] - Tissue: Human Fibroblasts Top: Before activation Bottom: After activation with poly IC Left: anti p65 NLS specific Right: Monoclonal antibody C-Term The two antibodies that are shown target different regions of the p65 protein. The different staining patterns are thought to correspond with different functional regions of the protein.

Immunohistochemistry: ReIA/NFkB p65 Antibody (27F9.G4) [NBP1-77815] - Antibody 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 2.5ug/ml. The image displayed shows specific staining in colon carcinoma as the precipitated red signal, with a hematoxylin purple nuclear counterstain.

Western Blot: RelA/NFkB p65 Antibody (27F9.G4) [NBP1-77815] -RelA/NFkB p65 antibody (27F9.G4) was used to detect ~65 kD band (red arrow) in HeLa whole cell lysate. 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 at 4C and detected with HRP conjugated Rbanti-Mouse antibody at 1:40,000 in for 30 min at RT.



65 kD -





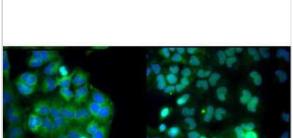


Immunocytochemistry/Immunofluorescence: ReIA/NFkB p65 Antibody (27F9.G4) [NBP1-77815] - Used at a dilution of 1:5000. Hela cells were grown to sub-confluent on 18 mm2 glass coverslips #1.5. Cells were either unstimulated (A), or stimulated (B) with 50 ng/ml of TNF alpha for 30 min prior fixation. Cells 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.

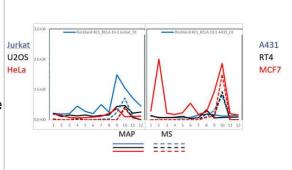
Immunocytochemistry/Immunofluorescence: ReIA/NFkB p65 Antibody (27F9.G4) [NBP1-77815] - Mouse endothelial cells. Unstimulated control cells (left) show cytoplasmic staining, TNF-alpha stimulated cells (right) show nuclear staining. For staining, cells were washed with PBS to remove all traces of culture 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.

Immunocytochemistry/Immunofluorescence: ReIA/NFkB p65 Antibody (27F9.G4) [NBP1-77815] - ReIA/NFkB p65 antibody (27F9.G4) 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.

PAGE-MAP (microsphere affinity proteomics) of ReIA/NFkB p65 Antibody (27F9.G4). (Catalog Number: , 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.



100 µm





Publications

Erdogan S, Doganlar O, Doganlar ZB et al. The flavonoid apigenin reduces prostate cancer CD44(+) stem cell survival and migration through PI3K/Akt/NF-kB signaling. Life Sci. 2016-08-25 [PMID: 27569589] (WB)

Merkhofer EC, Cogswell P, Baldwin AS et al. Her2 activates NF-kappaB and induces invasion through the canonical pathway involving IKKalpha. Oncogene 2010-02-01 [PMID: 19946332]

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HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-96981-0.5mg	Mouse IgG2a Kappa Isotype Control (M2AK)
NBP1-77815UV	ReIA/NFkB p65 Antibody (27F9.G4) [DyLight 350]

Limitations

This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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