

NFkB p65 Polyclonal Antibody

Product Details

Size	100 µg
Species Reactivity	Human, Mouse
Published Species	Mouse, Human
Host/Isotype	Rabbit / IgG
Class	Polyclonal
Type	Antibody
Conjugate	Unconjugated
Immunogen	GLLSGDEDFSSIADMDFS
Form	Liquid
Concentration	1 mg/mL
Purification	Antigen affinity chromatography
Storage buffer	PBS with 30% glycerol, 1mg/mL BSA
Contains	0.05% sodium azide
Storage conditions	-20°C
RRID	AB_2539917

Applications	Tested Dilution	Publications
Western Blot (WB)	1-3 µg/mL	9 Publications
Immunocytochemistry (ICC/IF)	1-2 µg/mL	4 Publications
Flow Cytometry (Flow)	3-5 µg/1x10 ⁶ cells	-
Immunoprecipitation (IP)	3 µg	-
ChIP assay (ChIP)	1-5 µg x 10 ⁶ cells	-

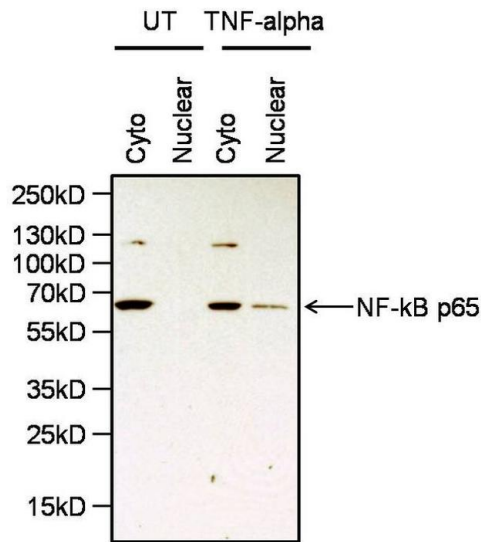
Product Specific Information

PA1-186 has been successfully used in WB using StartingBlock T20 (TBS) Blocking Buffer (Product # 37543). More non-specific bands are observed when using 5% BSA for blocking.

By WB, PA1-186 detects a predominant band at 65kD. Loading increasing amounts of cell lysate may yield more intense nonspecific bands at ~120kD and/or ~50kD.

PA1-186 has been successfully used for IP of the NF-kB p65 subunit and co-IP of the NF-kB p50 subunit.

Product Images For NFkB p65 Polyclonal Antibody

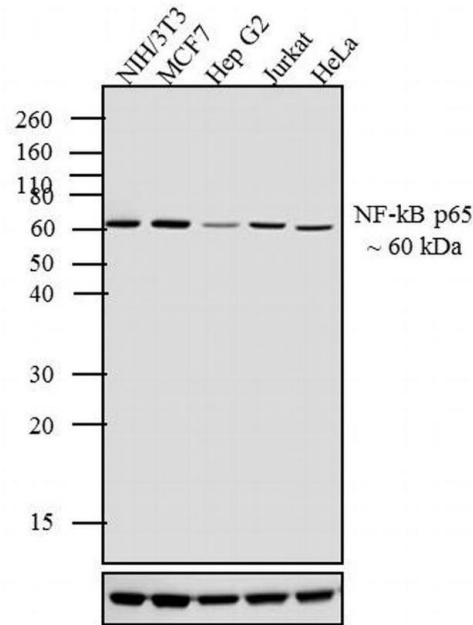


NFkB p65 Antibody (PA1-186)

Modulation of expression of target protein by cell treatment to demonstrate antibody specificity. Western blot analysis of NF-kB p65 using NFkB p65 Polyclonal Antibody (Product # PA1-186) shows enrichment of NF-kB p65 in the nuclear fraction of HeLa cells upon treatment with TNF-alpha, as compared to untreated cells. {TM}

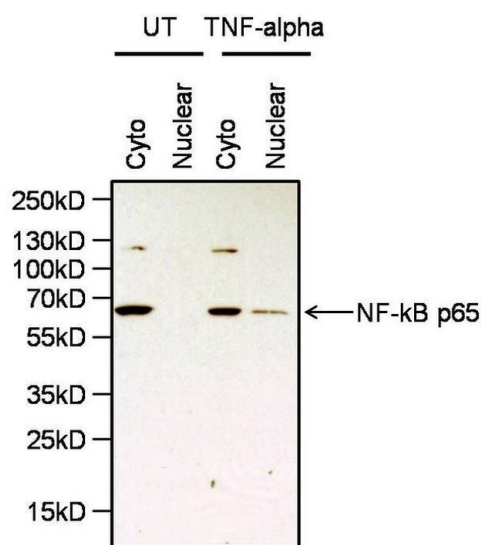
NFkB p65 Antibody (PA1-186) in WB

Western blot analysis was performed on whole cell extracts (20 µg lysate) of NIH /3T3 (Lane 1), MCF7 (Lane 2), Hep G2 (Lane 3), Jurkat (lane 4) and HeLa (lane 5). The blots were probed with Anti-NF-kB p65 Rabbit Polyclonal Antibody (Product # PA1-186, 1-3 µg/mL) and detected by chemiluminescence using Goat anti-Rabbit IgG (Heavy Chain) Superclonal™ Secondary Antibody, HRP conjugate (Product # A27036, 0.4 µg/mL, 1:2500 dilution). A 60 kDa band corresponding to NF-kB p65 was observed across cell lines tested. Known quantity of protein samples were electrophoresed using Novex® NuPAGE® 4-12 % Bis-Tris gel (Product # NP0321BOX), XCell SureLock™ Electrophoresis System (Product # EI0002) and Novex® Sharp Pre-Stained Protein Standard (Product # LC5800). Resolved proteins were then transferred onto a nitrocellulose membrane with iBlot® 2 Dry Blotting System (Product # IB21001). The membrane was probed with the relevant primary and secondary Antibody following blocking with 5 % skimmed milk. Chemiluminescent detection was performed using Pierce™ ECL Western Blotting Substrate (Product # 32106).



NFkB p65 Antibody (PA1-186) in WB

Western blot analysis of NF-kB p65 was performed by loading 20 µg of cytoplasmic (cyto) and nuclear extracts from HeLa cells either left untreated (UT) or treated with 25 ng/mL of recombinant TNF-alpha (Product # RTNFAI) for 35 minutes at 37C, and 10 µL of PageRuler Plus Prestained Protein Ladder (Product # 26619) per well onto a 4-20% Tris-HCl polyacrylamide gel. Proteins were transferred to a PVDF membrane (Product # 88518) using the G2 Fast Blotter (Product # 62288), and blocked with StartingBlock T20 (TBS) Blocking Buffer (Product # 37543) for at least 1 hour at room temperature. NF-kB p65 was detected at 65 kD using an NF-kB p65 polyclonal antibody (Product # PA1-186) at a dilution of 1:2000 in StartingBlock T20 (TBS) Blocking Buffer overnight at 4C on a rocking platform, followed by an HRP-conjugated goat anti-rabbit IgG secondary antibody (Product # 31460) at a dilution of 1:40,000 for 30 minutes at room temperature. Chemiluminescent detection was performed using SuperSignal West Pico (Product # 34080).



13 References

Western Blot (9)

<div>Biomedicines</div> <div>SIRT1-Dependent Upregulation of BDNF in Human Microglia Challenged with A: An Early but Transient Response Rescued by Melatonin.</div> <div>"PA1-186 was used in Western Blotting to define an early microglial defensive response to A42, featuring SIRT1-mediated BDNF upregulation that can be exogenously modulated by melatonin, thus identifying an important target for neuroprotection."</div> <div>Authors: Caruso GI,Spampinato SF,Costantino G,Merlo S,Sortino MA</div>	<div>Year</div> <div>2021</div> <div>Species</div> <div>Human</div> <div>Dilution</div> <div>1:400</div>
<div>Biomolecules</div> <div>SIRT1 Mediates Melatonin's Effects on Microglial Activation in Hypoxia: In Vitro and In Vivo Evidence.</div> <div>"PA1-186 was used in Western Blotting to provide new evidence for a direct effect of melatonin on hypoxic microglia through SIRT1, which appears as a potential pharmacological target against hypoxic-derived neuronal damage."</div> <div>Authors: Merlo S,Luaces JP,Spampinato SF,Toro-Urrego N,Caruso GI,D'Amico F,Capani F,Sortino MA</div>	<div>Year</div> <div>2020</div> <div>Species</div> <div>Mouse</div> <div>Dilution</div> <div>1:400</div>

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Immunocytochemistry (4)

<div>Molecular neurobiology</div> <div>Selective GSK3 Inhibition Mediates an Nrf2-Independent Anti-inflammatory Microglial Response.</div> <div>"PA1-186 was used in Immunocytochemistry-immunofluorescence to explore a multimodal disease-modifying utility of GSK3 inhibition, beyond neuronal proteopathologies. Furthermore, we aimed to underscore the difference in therapeutic value between the two GSK3 paralogs by isoform-selective chemical inhibition."</div> <div>Authors: Yousef MH,Salama M,El-Fawal HAN,Abdelnaser A</div>	<div>Year</div> <div>2022</div> <div>Species</div> <div>Mouse</div>
<div>International journal of molecular sciences</div> <div>Sex and APOE Genotype Alter the Basal and Induced Inflammatory States of Primary Microglia from APOE Targeted Replacement Mice.</div> <div>"Published figure using NFkB p65 polyclonal antibody (Product # PA1-186) in Immunocytochemistry"</div> <div>Authors: Mhatre-Winters I,Eid A,Han Y,Tieu K,Richardson JR</div>	<div>Year</div> <div>2022</div>

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