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	-

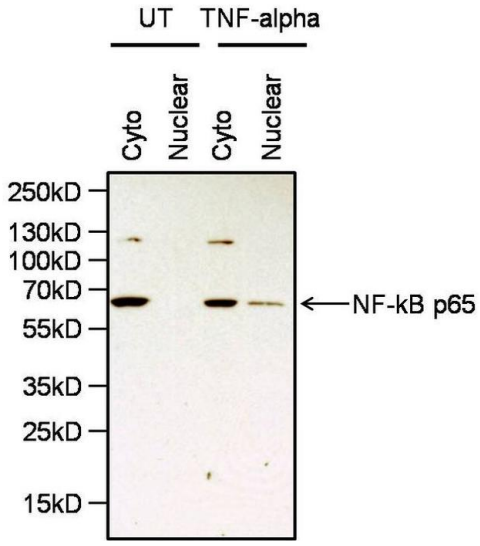
Product Specific Information

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.

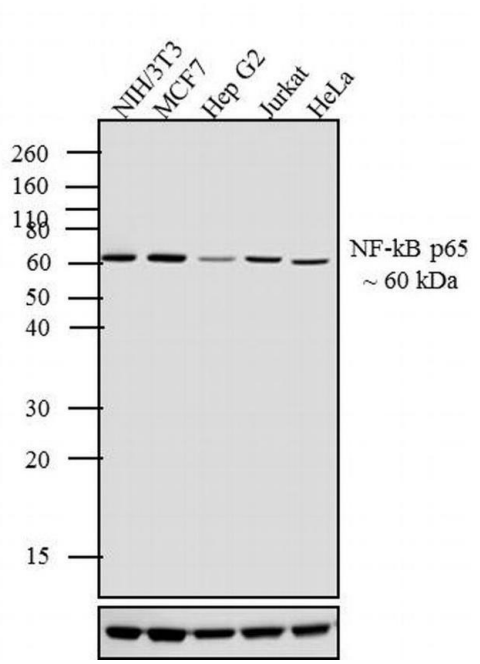
By WB, PA1-186 detects a predominant band at 65kD. Loading increasing amounts of cell lysate may yield more intense nonspecific bands at ~120kD and/or ~50kD.

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

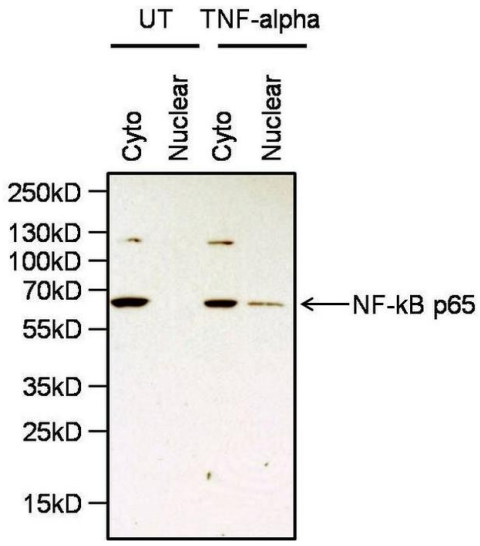
Product Images For NFkB p65 Polyclonal Antibody



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).

View more figures on thermofisher.com

13 References

Western Blot (9)

Biomedicines

SIRT1-Dependent Upregulation of BDNF in Human Microglia Challenged with A: An Early but Transient Response Rescued by Melatonin.

"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."

Authors: Caruso GI,Spampinato SF,Costantino G,Merlo S,Sortino MA

Year 2021

Species Human

Dilution 1:400

Biomolecules

SIRT1 Mediates Melatonin's Effects on Microglial Activation in Hypoxia: In Vitro and In Vivo Evidence.

"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."

Authors: Merlo S,Luaces JP,Spampinato SF,Toro-Urrego N,Caruso GI,D'Amico F,Capani F,Sortino MA

Year 2020

Species Mouse

Dilution 1:400

View more WB references on thermofisher.com

Immunocytochemistry (4)

Molecular neurobiology

Selective GSK3 Inhibition Mediates an Nrf2-Independent Anti-inflammatory Microglial Response.

"PA1-186 was used in Immunocytochemistry-immunoflourescence 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."

Authors: Yousef MH,Salama M,El-Fawal HAN,Abdelnaser A

Year 2022

Species Mouse

International journal of molecular sciences

Sex and APOE Genotype Alter the Basal and Induced Inflammatory States of Primary Microglia from APOE Targeted Replacement Mice.

"Published figure using NFkB p65 polyclonal antibody (Product # PA1-186) in Immunocytochemistry"

Authors: Mhatre-Winters I,Eid A,Han Y,Tieu K,Richardson JR

Year 2022

View more ICC/IF references on thermofisher.com

More applications with references on thermofisher.com