

Anti-NF-kB p65/RELA Antibody

Catalog Number: PA1669

About RELA

RELA (V-REL AVIAN RETICULOENDOTHELIOSIS VIRAL ONCOGENE HOMOLOG A), also called NFKB3 or NFKB, p65 SUBUNIT. NFKB1 or NFKB2 is bound to REL, RELA, or RELB to form the NFKB complex. The NFKB complex is inhibited by I-kappa-B proteins, which inactivate NFKB by trapping it in the cytoplasm. The p65 (RELA) heterodimer is the most abundant form of NFKB. And the RELA gene is located on 11q13.1. RELA is a nonhistone substrate of HDAC3 and that IKBA-dependent nuclear export of the HDAC3-deacetylated RELA replenishes the depleted cytoplasmic pool of latent NFKB-IKBA complexes for subsequent NFKB responses. RELA nucleocytoplasmic redistribution coincided with export of PPARG, and immunoprecipitation analysis indicated that PPARG-RELA association was dependent on the PPARG C-terminal ligand-binding domain. IKK-dependent phosphorylation of RELA on ser468 enhanced binding of GCN5 to RELA and RELA ubiquitination.

Overview

Product Name	Anti-NF-kB p65/RELA Antibody
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-NF-kB p65/RELA Antibody catalog # PA1669. Tested in IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 5mg BSA, 0.9mg NaCl, 0.2mg Na2HPO4, 0.05mg Thimerosal, 0.05mg NaN3.
Storage Instructions	Store at -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	Q04206

Technical Details

Immunogen	A synthetic peptide corresponding to a sequence in the middle region of human NF-kB p65, identical to the related rat and mouse sequences.
Predicted Reactive Species	Bovine
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti-Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG

Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	<p>Dilute the sample so that the expected range of concentrations fall within the detection range of this kit.</p> <p>If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples.</p> <p>Some PubMed article(s) citing the expression level of this target are as follows:</p> <p>Boster Bio's internal QC testing used:</p> <p>Western blot, 0.1-0.5ug/ml, Human, Mouse, Rat</p> <p>Immunohistochemistry (Paraffin-embedded Section), 0.5-1ug/ml, Human, By Heat</p> <p>Immunocytochemistry/Immunofluorescence , 5ug/ml, Human, -</p>

Anti-NF- κ B p65/RELA Antibody (PA1669) Images

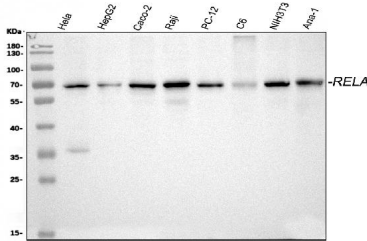


Figure 1. Western blot analysis of NF- κ B p65 using anti-NF- κ B p65 antibody (PA1669). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 μ g of sample under reducing conditions.

Lane 1: human Hela whole cell lysates,
Lane 2: human HepG2 whole cell lysates,
Lane 3: human Caco-2 whole cell lysates,
Lane 4: human Raji whole cell lysates,
Lane 5: rat PC-12 whole cell lysates,
Lane 6: rat C6 whole cell lysates,
Lane 7: mouse NIH/3T3 whole cell lysates,
Lane 8: mouse Ana-1 whole cell lysates.

After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-NF- κ B p65 antigen affinity purified polyclonal antibody (Catalog # PA1669) at 0.5 μ g/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for NF- κ B p65 at approximately 65 kDa. The expected band size for NF- κ B p65 is at 60 kDa.

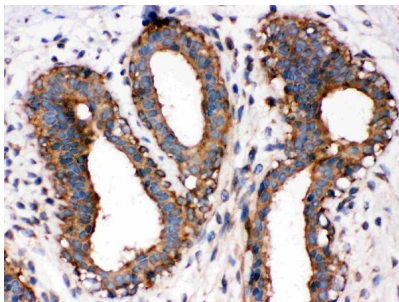


Figure 2. IHC analysis of NF- κ B p65 using anti-NF- κ B p65 antibody (PA1669).

NF- κ B p65 was detected in a paraffin-embedded section of Human Mammary Cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 1 μ g/ml rabbit anti-NF- κ B p65 Antibody (PA1669) overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit (Catalog # SV0002) with DAB as the chromogen.

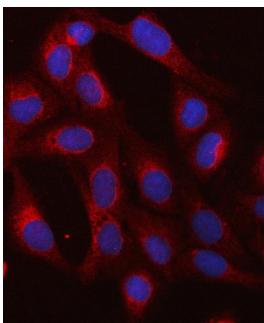


Figure 3. IF analysis of NF- κ B p65 using anti-NF- κ B p65 antibody (PA1669).

NF- κ B p65 was detected in an immunocytochemical section of U2OS cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 μ g/mL rabbit anti-NF- κ B p65 Antibody (PA1669) overnight at 4°C. Cy3 Conjugated Goat Anti-Rabbit IgG (BA1032) was used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. The section

was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

54 Publications Citing This Product

1. PubMed ID: 32417325, Wang F, Ji S, Wang M, Liu L, Li Q, Jiang F, Cen J, Ji B. HMGB1 promoted P-glycoprotein at the blood-brain barrier in MCAO rats via TLR4/NF-kappaB signaling pathway. Eur J Pharmacol. 2020 Aug 5;880:173189. doi:10.1016/j.ejphar.2020.173189. Epub 2020 May 15. PMID:32417325.
2. PubMed ID: 32794226, Ma G, Kimatu BM, Yang W, Pei F, Zhao L, Du H, Su A, Hu Q, Xiao H. Preparation of newly identified polysaccharide from Pleurotus eryngii and its anti-inflammation activities potential. J Food Sci. 2020 Sep;85(9):2822-2831. doi:10.1111/1750-3841.15375. Epub 2020 Aug 14.
3. PubMed ID: 32565851, Wang Y, Yang Z, Yang L, Zou Q, Zhao S, Hu N, Chen D, Cui R, Ma H. Liuweidihuang Pill Alleviates Inflammation of the Testis via AMPK/SIRT1/NF-kappaB Pathway in Aging Rats. Evid Based Complement Alternat Med. 2020 May 24;2020:2792738. doi:10.1155/2020/2792738. PMID:32565851.

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