BOSTER
antibody and ELISA experts

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Anti-NF-kB p65/RELA Antibody Picoband®

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 Picoband®
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. The brand Picoband indicates this is a premium antibody that guarantees superior quality, high affinity, and strong signals with minimal background in Western blot applications. Only our best-performing antibodies are designated as Picoband, ensuring unmatched performance.
Application	IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.
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.
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

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Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.1-0.5ug/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human Immunocytochemistry/Immunofluorescence , 5ug/ml, Human, -

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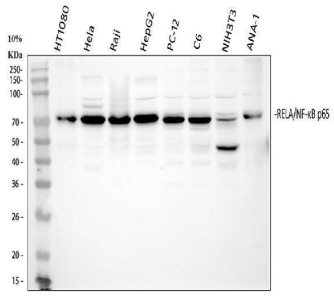
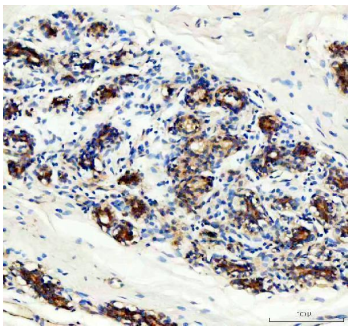
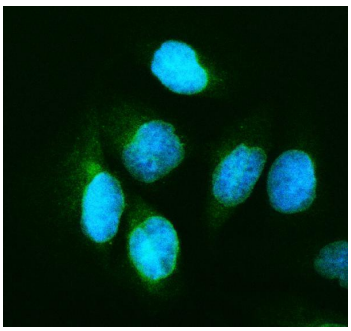
Anti-NF-kB p65/RELA Antibody Picoband® (PA1669) Images



Western blot analysis of NF-kB p65/RELA using anti-NF-kB p65/RELA 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 ug of sample under reducing conditions. Lane 1: human HT1080 whole cell lysates, Lane 2: human Hela whole cell lysates, Lane 3: human Raji whole cell lysates, Lane 4: human HepG2 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-kB p65/RELA antigen affinity purified polyclonal antibody (Catalog # PA1669) at 0.5 ug/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-kB p65/RELA at approximately 65-70 kDa. The expected band size for NF-kB p65/RELA is at 60 kDa.



IHC analysis of NF-kB p65/RELA using anti-NF-kB p65/RELA antibody (PA1669). NF-kB p65/RELA was detected in a paraffin-embedded section of human breast 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 2 ug/ml rabbit anti-NF-kB p65/RELA 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.



IF analysis of NF-kB p65/RELA using anti-NF-kB p65/RELA antibody (PA1669). NF-kB p65/RELA was detected in an immunocytochemical section of U20S 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 ug/mL rabbit anti-NF-kB p65/RELA 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.

Andro targets TLR4/NF-kappaB signaling in insulinoma.

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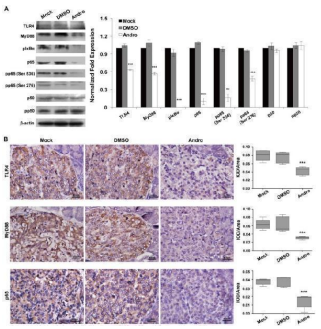
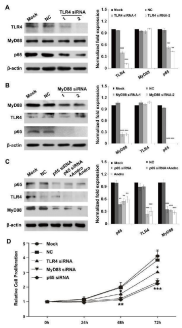
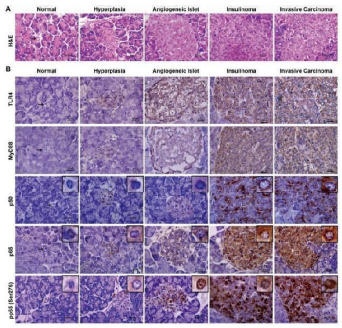


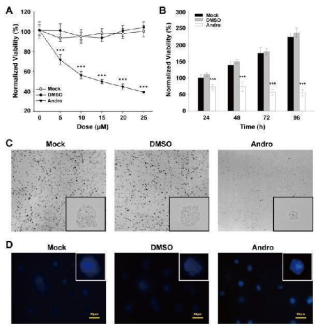
Andro can significantly inhibited the protein expression of TLR4, MyD88, phosphorylated IkappaBalpha, p65, phosphorylated p65 (Ser536), and phosphorylated p65 (Ser276) of TLR4/NF-kappaB signaling pathway in tumor tissues of RIP1-Tag2 mice compared with control groups. Immunohistochemical staining showed that Andro also inhibited the expression of TLR4 (B), MyD88 (C) and p65 (D) in tumor tissues of RIP1-Tag2 mice compared with control groups. Bar, 20 um. *** P < 0.001.Index in PubMed under a CC BY license. PMID: 24719558



TLR4/NF-kappaB signaling pathway is the dominant functional target of andrographolide . (A) TLR4 deficient could only slightly inhibit the expression of p65 and cannot suppress the expression of MyD88. (B) The inhibition of MyD88 result the reduction of p65 but not TLR4 (C) p65 deficient could inhibit the expression of MyD88 but not TLR4. (D) The inhibition of TLR4, MyD88 and p65 can both suppressed cell proliferation. n=12, * P < 0.05, ** P < 0.01 and *** P < 0.001.Index in PubMed under a CC BY license. PMID: 24719558



The expression and activation of TLR4/NF-kappaB signaling is increased during insulinoma development. (A) The classification of insulinoma. The H&E staining defined the stages of insulinoma. (B) The immunohistochemical staining of TLR4, MyD88, p50, p65 and phosphorylated p65 (Ser276) at the stage of normal, hyperplasia, angiogenic islet, insulinoma, and invasive carcinoma in RIP1-Tag2 mice. Results are representative of at least 3 tissue samples in a from more than 3 mice for each stage. Bar, 20 um.Index in PubMed under a CC BY license. PMID: 24719558



Andro suppresses cell proliferation and clonogenicity and induces cell apoptosis in beta-TC-6 cells. (A) The cells were treated with various doses of Andro for 48 hours, and the cell viability was quantified by MTT assay. (B) The cells were treated with the IC 50 dose of Andro, and relative cell viability was measured using the MTT assay at the indicated times. (C) The clonogenicity ability of beta-TC-6 was significantly suppressed by Andro compared with control groups. Representative photomicrographs are shown. (D) Andro induces cell apoptosis in beta-TC-6 cells that treated with the IC 50 dose of Andro for 48 h. Cells were stained with Hoechst 33342 and apoptotic cells were identified by condensation and fragmentation of nuclei using inverted light microscope. All values are presented for the mean ± s.d., n=12; *** P < 0.001.Index in PubMed under a CC BY license. PMID: 24719558

Red nucleus IL-33 facilitates the early development of mononeuropathic pain by activating NF-kappaB signaling pathway. A Western blotting showed an upregulated NF-kappaB in the RN at 1 week post-SNI, intrarubral administration of anti-IL-33 antibody restrained the

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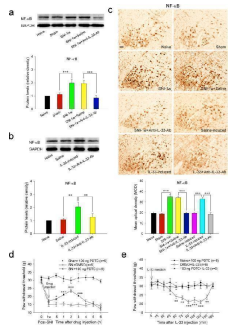
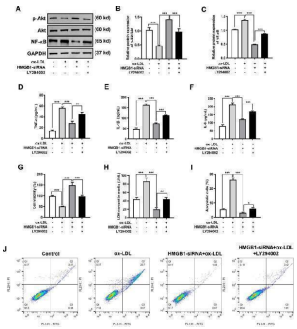
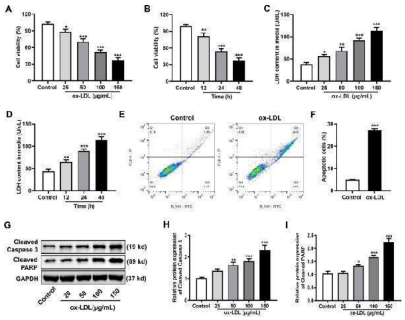


overexpression of NF-kappaB (n = 6 per group, F = 12.766, P < 0.001). B Western blotting showed that intrarubral injection of IL-33 stimulated the protein expression of NF-kappaB in naive rats (n = 6 per group, F = 7.497, P = 0.003). C Immunohistochemistry demonstrated that NF-kappaB was increased in the RN of SNI rats (F = 41.250, P < 0.001) and IL-33-induced hypersensitivity rats (F = 34.509, P < 0.001) (n = 4 per group). D Intrarubral injection of NF-kappaB inhibitor PDTC at 1 week post-injury attenuated SNI-induced mononeuropathic pain compared to DMSO control (n = 5–6 per group, F = 135.298, P < 0.001). E PDTC pre-injected into the RN, 30 min ahead of IL-33 administration, relieved IL-33-evoked mechanical hypersensitivity compared to DMSO control (n = 5-6 per group, F = 97.341, P < 0.001). * P < 0.05, ** P < 0.01, and *** P < 0.001. Scale bars = 50 um Index in PubMed under a CC BY license. PMID: 34225736



Inhibition of PI3K-Akt pathway promoted the role of HMGB1. HUVECs were pretreated with 10 uM LY294002 for 1 h. A Representative protein bands showing the expression of Akt, Akt phosphorylation, and NF-kappaB in HUVECs. GAPDH served as a loading control. Original images of western blots were shown in Additional file : Supplementary Fig. 1I–K. B The protein quantification ratio of P-Akt/Akt. C Relative protein expression levels of NF-kappaB. Quantitation of D TNF-alpha, E IL-1beta, and F IL-6 in the supernatant of HUVECs was performed by ELISA. G Cell viability in each group detected using a CCK8 assay. H Cytotoxicity detected using a LDH kit. I The percentage of apoptotic cells in each group in J was calculated. J Representative flow cytometry profiles of different treatment groups. '+' was added, '−' was blank. * p



Changes in viability and apoptosis rate of HUVECs during ox-LDL-induced damage. A Cell viability after treatment with different concentrations of ox-LDL (25, 50, 100, or 150 ug/mL) detected using a CCK8 kit. B Cell viability at different time points (12, 24, or 48 h) when HUVECs were cultured with 100 ug/mL ox-LDL. C Cell cytotoxicity after treatment with different concentrations of ox-LDL detected using a LDH kit. D Cell cytotoxicity at different time points when HUVECs were cultured with 100 ug/mL ox-LDL. E Representative flow cytometry profiles of the control and ox-LDL groups. The HUVECs in the control group were untreated and the ox-LDL group was treated with 100 ug/mL ox-LDL for 24 h. F Percentage of apoptotic cells. G Representative western blot images of Cleaved Caspase-3 and Cleaved PARP expression. Original images of western blots are shown in Additional file : Supplementary Fig. 1A–C. The relative protein expression levels of H Cleaved Caspase-3 and I Cleaved PARP. Each experiment was independently repeated three times and the data were expressed as means ± SEM. * p

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

Anti-NF-kB p65/RELA Antibody

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