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Anti-NF-kB p65/RELA Antibody Picoband®

Catalog Number: A00284-1

About RELA

Transcription factor p65, also known as NFKB3 or NF-kB p65, is a protein that in humans is encoded by the RELA gene. It is mapped to 11q13.1. NFKB is an essential transcription factor complex involved in all types of cellular processes, including cellular metabolism, chemotaxis, etc, and it may play a role in inflammatory conditions of the peripheral nervous system. Phosphorylation and acetylation of NFKB3 are crucial post-translational modifications required for NFKB activation. It has also been shown to modulate immune responses, and activation of NFKB3 is positively associated with multiple types of cancer. In addition to that, NFKB3 antagonizes TNFR1-JNK proliferative signals in epidermis and plays a nonredundant role in restraining epidermal growth.

Overview

Product Name	Anti-NF-kB p65/RELA Antibody Picoband®
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-NF-kB p65/RELA Antibody Picoband® catalog # A00284-1. Tested in ELISA, Flow Cytometry, IF, 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	ELISA, Flow Cytometry, IF, 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	E. coli-derived human NF-kB p65 recombinant protein (Position: F99-S551).
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 ICC.
Cross Reactivity	No cross-reactivity with other proteins.
Isotype	Rabbit IgG
Form	Lyophilized

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Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Western blot, 0.1-0.5 ug/ml Immunocytochemistry/Immunofluorescence, 5 ug/ml Immunoprecipitation, 0.5-2 ug/ml Flow Cytometry (Fixed), 1-3ug/1x10^6 cells ELISA, 0.1-0.5 ug/ml

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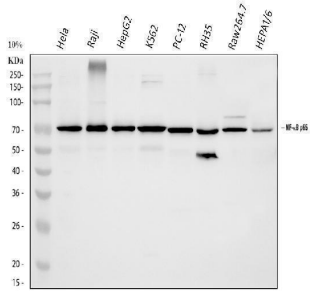
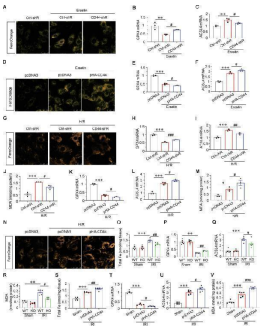
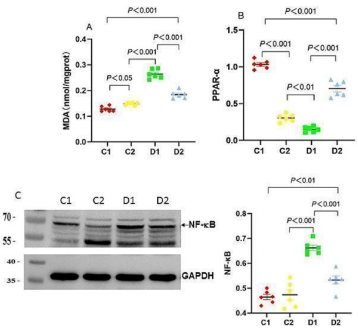
Anti-NF-kB p65/RELA Antibody Picoband® (A00284-1) Images



Western blot analysis of NF-kB p65 using anti-NF-kB p65 antibody (A00284-1). 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 Hela whole cell lysates, Lane 2: human Raji whole cell lysates, Lane 3: human HepG2 whole cell lysates, Lane 4: human K562 whole cell lysates, Lane 5: rat PC-12 whole cell lysates, Lane 6: rat RH35 whole cell lysates, Lane 7: mouse RAW264.7 whole cell lysates, Lane 8: mouse HEPA1/6 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 antigen affinity purified polyclonal antibody (Catalog # A00284-1) 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 at approximately 70 kDa. The expected band size for NF-kB p65 is at 60 kDa.



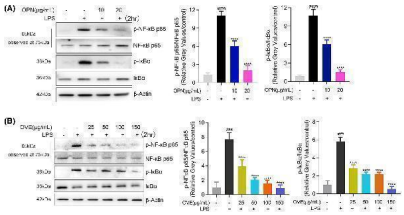
CD44 promotes tubular cell injury and AKI partially through ferroptosis. A HKC-8 was transfected with Ctrl-shR or CD44-shR plasmid and then treated with 5 uM erastin for 24 h. Representative micrographs show the Fe 2+ content via FerroOrange staining in different groups, as indicated. Scale bar, 25 um. B and C Quantitative results of QPCR showing relative (B) GPX4 and (C) ACSL4 mRNA levels among different groups. ** P



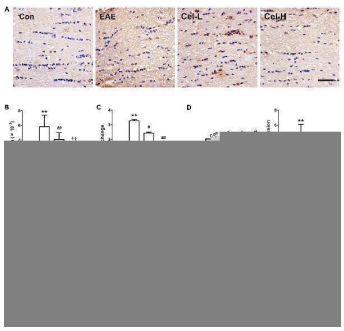
Effects of n-6 PUFA on liver lipid peroxidation and the inflammatory marker NF-kappaB in rats with NASH induced by a choline-deficient diet. (A) Liver MDA levels, (B) PPAR-alpha mRNA expression in the liver. Data are expressed as mean ±SEM; n = 6/group. (C) NF-kappaB protein expression (~65 kDa) in the liver as analyzed by Western blotting, normalized to GAPDH, with a representative blot (left) and quantification (right). Protein molecular weight standards (kDa) are labeled on the left of each blot. Data are expressed as mean ± SEM; n = 6/group.Index in PubMed under a CC BY license. PMID: 40626231

Effects of n-6 PUFA on liver macrophage phenotype in rats with NASH induced by a choline-deficient diet. (A) M1-type Kupffer cells (KCs) identified by double staining: red arrows show CD11c-positive cells, green arrows show CD68-positive

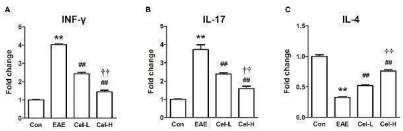
cells, and yellow arrows highlight CD11c and CD68 double-positive M1-type KCs (Scale bar – 50 uM). (B) M2-type KCs identified similarly, with red arrows indicating CD163-positive cells, green arrows showing CD68-positive cells, and yellow arrows marking CD163 and CD68 double-positive M2-type KCs (Scale bar – 50 uM). For (A,B) (see) for full-size photomicrographs. (C) M1/M2 phenotype ratio (unitless), calculated as the proportion of CD68 + CD11c + to CD68 + CD163 + cells. (D) Relative PPAR-gamma2 mRNA expression (fold change normalized to GAPDH) in the liver, which is linked to macrophage polarization and inflammation. Data are expressed as mean ±SEM; n = 6/group.Index in PubMed under a CC BY license. PMID: 40626231



Effects of OPN and OVE on activation of NF-κB pathways in LPS-stimulated RAW264.7 cells. Cells were pretreated with OVE or OPN at different concentrations for 1 h. Expression levels of p-NF-κB p65, NF-κB p65, p-IκBalpha, and IκBalpha were detected after 24 h of LPS treatment. (A) OPN treatment. (B) OVE treatment. All experiments were carried out in triplicates and data are presented as means ± SDs; one-way ANOVA analysis was adopted for multiple comparisons; ###P<0.001, ####P<0.0001, compared to the untreated control group; ****P<0.0001, compared to the LPS control group. Index in PubMed under a CC BY license. PMID: 39455284



Celastrol inhibited inducible nitric oxide synthase (iNOS) expression and activation of NF-kappaB in optic nerve in EAE rats. (A) IHC staining of iNOS in optic nerve. (B) Quantification of iNOS-positive areas. (C) Quantitative real-time PCR analysis of iNOS expression. (D) Western blot analysis of iNOS expression. (E–G) Western blot analysis of IkappaBalpha, p65 and p-p65 expression, respectively. Treatment of celastrol reduced expression of iNOS and inhibited the activation of NF-kappaB in optic nerve in EAE rats. Scale bar: 100 um. Data were shown as mean ± SD, n = 5. ∗∗ P < 0.01 versus control group, ## P < 0.01 versus EAE group, †† P < 0.01 versus low dosage of celastrol group.Index in PubMed under a CC BY license. PMID: 28239352



Celastrol regulated production of cytokines and activation of NF-kappaB in spinal cords of EAE rats. Celastrol dose-dependently downregulated the mRNA expression of INF-gamma (A) and IL-17 (B) but upregulated IL-4 (C) in spinal cord of EAE rats. Data were shown as mean ± SD, n = 5. ∗∗ P < 0.01 versus control group, ## P < 0.01 versus EAE group, †† P < 0.01 versus low dosage of celastrol group.Index in PubMed under a CC BY license. PMID: 28239352

CD44 induces tubular cell injury through MAPK-NF-kappaB p65-silenced PGC-1alpha signaling. A and B HKC-8 was transfected with pcDNA3 or p-Flag-p65 overexpression

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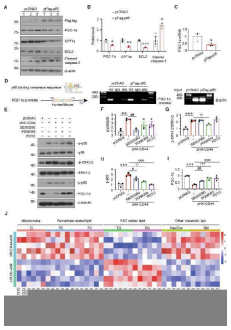
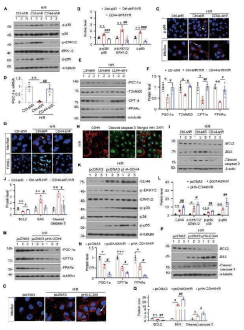
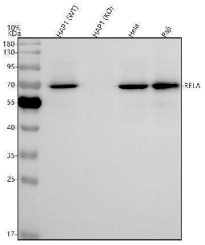


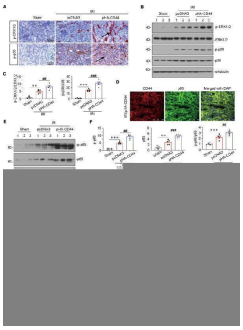
plasmid for 24 h. Representative western blot (A) and graphical presentations of PGC-1alpha, CPT1a, BCL2, and cleaved caspase 3 protein expression levels are shown. * P



CD44 aggravates mitochondrial and FAO dysfunction and drives cell apoptosis through MAPK and NF-kappaB p65 signaling in vitro. A and B HKC-8 was transfected with Ctrl-shR or CD44-shR and then were incubated in basal culture medium in a 1% O 2 environment for 24 h and then were reoxygenated in normal O 2 for 6 h. Representative western blot (A) and graphical presentations of p-p38/p38, p-ERK1/2/ERK1/2, and p-p65 protein expression levels are shown. ** P



Western blot analysis of NF-kB p65 using anti-NF-kB p65 antibody (A00284-1). 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 HAP1-WT whole cell lysates, Lane 2: human HAP1-NF-kB p65 KO whole cell lysates, Lane 3: human Hela whole cell lysates, Lane 4: human Raji 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 antigen affinity purified polyclonal antibody (Catalog # A00284-1) 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 at approximately 70 kDa. The expected band size for NF-kB p65 is at 60 kDa.



Ectopic expression of CD44 impairs mitochondrial function and FAO through activating MAPK and NF-kappaB p65 signaling. A Representative micrographs show the expression of p-ERK1/2 and p-p38 in different groups, as indicated. Paraffin sections were stained with antibodies against p-ERK1/2 and p-p38. Arrows indicate positive staining. Scale bar, 50 um. B and C Representative western blot (B) and graphical presentations of p-ERK1/2/ERK1/2 and p-p38/p38 protein levels are shown. ** P

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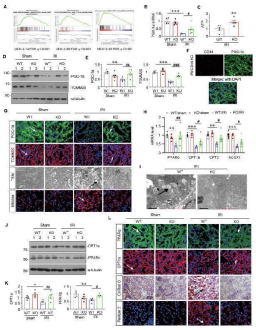
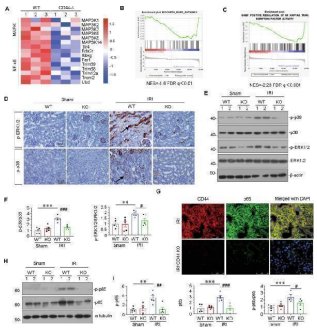
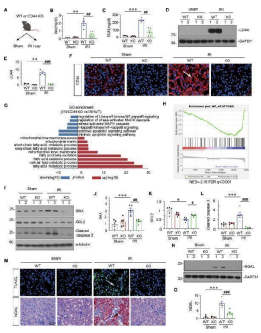


CD44 knockout ameliorates mitochondrial dysfunction and FAO deficiency in IRI mice. A GSEA shows that positive regulation of mitochondrial function and FAO was enriched in CD44 knockout mice versus wild-type mice upon IRI. NES, normalized enrichment score; FDR q -value<0.25. B Graphic presentation shows the relative levels of renal expression of PGC-1alpha mRNA in different groups as indicated. *** P



CD44 promotes AKI progression through inducing MAPK and NF-kappaB p65 signaling. A Representative heatmap gene expression of RNA sequencing analysis shows that CD44 is involved with MAPK and NF-kappaB signaling pathway. B and C GSEA shows that negative regulation of MAPK and NF-kappaB pathway was enriched in CD44 knockout mice versus wild-type mice upon IRI. NES, normalized enrichment score; FDR q -value<0.25. D Representative micrographs show the expression of p-ERK1/2 and p-p38 in different groups, as indicated. Paraffin sections were stained with antibodies against p-ERK1/2 and p-p38. Arrows indicate positive staining. Scale bar, 50 um. E and F Representative western blot (E) and graphical presentations of p-p38/p38 and p-ERK1/2/ERK1/2 protein levels are shown. ** P

Western blot analysis of NF-kB p65 using anti-NF-kB p65 antibody (A00284-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 80V (Stacking gel) / 120V (Resolving gel) for 2 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1-5: mouse tissue, 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 p65antigen affinity purified polyclonal antibody (A00284-1) at 1:2000 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 (Catalog # BA1054) at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an ECL Plus Western Blotting Substrate (Catalog # AR1196-200) with Tanon 5200 system. A specific band was detected for NF-kB p65 at approximately 70 kDa. The expected band size for NF-kB p65 is at 70 kDa.



Gene ablation of CD44 attenuates renal tubular cell apoptosis and kidney injury upon IRI. A Experimental design: Wild-type mice and CD44 conventional knockout mice were subjected to IRI or sham, respectively, and euthanized 24 h after IRI. B Scr levels in four groups, as indicated. Scr was expressed as milligrams per deciliter. ** P

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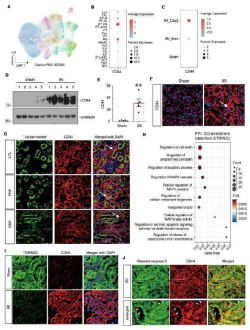
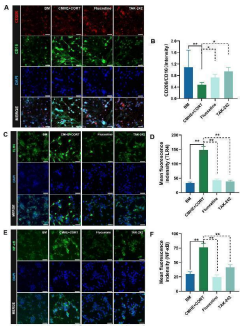
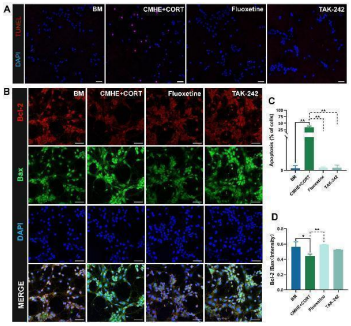


CD44 is upregulated in TECs and associated with mitochondrial dysfunction and apoptosis. A UMAP shows cell population in kidneys of sham and IRI at 6 h and day 2. PT proximal tubule, PT-Inj injured PT, PT-R repairing PT, FR-PTC failed repair PT cell, PT-AcInj acute injury PT, DTL descending limb of loop of Henle (LoH), ATL thin ascending limb of LoH, TAL thick ascending limb of LoH, DCT distal convoluted tubule, CNT connecting tubule, PC principal cell of collecting duct, ICA type A intercalated cell of collecting duct, ICB type B intercalated cell of collecting duct, Pod podocyte, EC endothelial cell, Fib fibroblast, Myofib myofibroblast, Ma macrophage (Mphi), B/T immune cell, Uro urothelium. Data from PMID: 36265491. B Graphic presentation of single-cell sequencing analysis shows the expression of CD44 in different cell populations. C Graphic presentation of single-cell sequencing analysis shows the expression of CD44 at different time point. D and E Representative western blot of CD44 (D) and graphical presentations (E) of protein expressional levels are shown. ** P



Activation of TLR4/NF-kappaB signaling and increased microglial M1-type polarization induces neuronal damage in the tri-culture model. (A) Fluorescence images of CD206 amd CD16 proteins in the microglia in the different groups. (B) Ratio of the relative fluorescence intensities between CD206 amd CD16 proteins in microglia. (C) Fluorescence images of TLR4 protein in neurons in the different groups. (D) Changes in fluorescence intensity of TLR4 protein in neurons in the different groups. (E) Fluorescence images of NF-kappaB protein in neurons in the different groups. (F) Changes in fluorescence intensity of NF-kappaB in neurons in the different groups. Data are presented as the mean ± SD (n = 3). * P < 0.05, ** P < 0.01 vs. BM group (one-way ANOVA followed by LSD or Tamhane’s T2). Scale bar = 50 um. BM: blank media; CHME: conditioned media of hypertensive environment; CORT: corticosterone; TLR4: toll-like receptor 4; NF-kappaB: nuclear factor-kappaB.Index in PubMed under a CC BY license. PMID: 40230380



TLR4 inhibitors suppress neuronal apoptosis. (A) Fluorescence images of TUNEL staining in the different groups (red, apoptotic cells; blue, nucleus). (B) The number of apoptotic cells in the different groups. (C) Fluorescence images of apoptosis-related proteins Bcl-2 amd Bax in the different groups. (D) Ratio of the relative fluorescence intensity between Bcl-2 and Bax proteins in the different groups. Data are presented as the mean ± SD (n = 3). * P < 0.05, ** P < 0.01 vs. BM group (one-way ANOVA followed by LSD or Tamhane’s T2). Scale bar = 50 um. BM: blank media; CHME: conditioned media of hypertensive environment; CORT: corticosterone; Bcl-2: B-Cell Lymphoma 2; Bax: Bcl 2-Associated X.Index in PubMed under a CC BY license. PMID: 40230380

Effects of HMGB1 down-regulation on NF-kappaB/p65,

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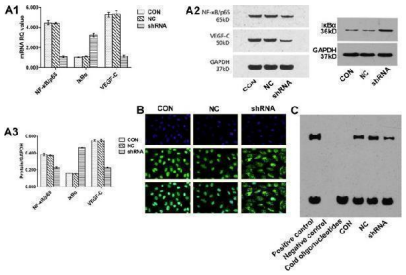
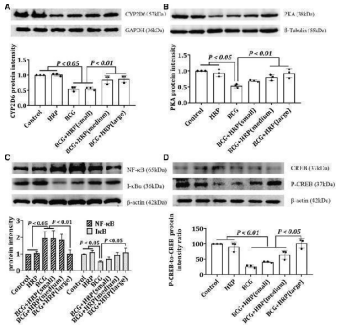
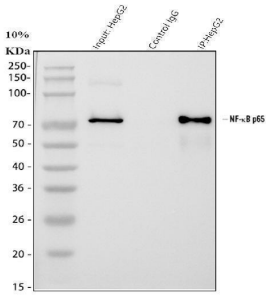


IkappaBalpha and VEGF-C in T24 cells. A1, A2 and A3: The expression of NF-kappaB/p65 and VEGF-C in the shRNA group was lower than in the other two groups transfected with shNC plasmids or the untransfected controls (all P 0.05). The display of cropped gels is used to improve the clarity and conciseness of the presentation and all the cropped gels have been run under the same experimental conditions. : The blue areas indicate nuclei stained using 4, 6-diamidino-2-phenylindole (DAPI) and the green areas indicate the nuclear translocation of NF-kappaB/p65 in T24 cells transfected with shNC plasmids or untransfected and cytoplasmic localization of NF-kappaB/p65 in cells transfected with shRNA plasmids. The results showed that knockdown of HMGB1 expression inhibited the translocation of NF-kappaB/p65 from the cytoplasm to the nucleus. : EMSA revealed that the DNA-binding activity of NF-kappaB/p65 in T24 cells was decreased by HMGB1 knockdown. Index in PubMed under a CC BY license. PMID: 26239046



Effect of HRP on the expression of CYP2D6, PKA, CREB, PCREB, IkappaB, and NF-kappaB in rats with BCG-induced immune-mediated liver injury. Rats were administered BCG (single intravenous dose of 125 mg/kg BCG) or BCG + HRP (50, 100, or 200 mg · kg −1 · d −1 orally for 7 d). Liver proteins were extracted to determine the expression levels of CYP2D6, PKA, CREB, PCREB, IkappaB, and NF-kappaB. SDS-PAGE was performed using equal amounts (30 ug) of protein, and western blotting was performed using antibodies against CYP2D6, PKA, CREB, PCREB, i-kappaB, and NF-kappaB. The results were normalized to tubulin, GAPDH, or beta-actin. The protein expression levels of CYP2D6 (a), PKA (b), IkappaB and NF-kappaB (c), CREB, and P-CREB (d) in the rat liver were measured by western blotting. The expression levels of CYP2D6, PKA, IkappaB, NF-kappaB, CREB, and P-CREB were quantified using the ImageJ software (NIH, Maryland, USA). The data represent the mean ± standard deviation (SD) of three independent experiments. BCG Bacille Calmette-Guerin; HRP Hippophae rhamnoides. Index in PubMed under a CC BY license. PMID: 37833431



Immunoprecipitating NF-kB p65 in HepG2 whole cell lysate. Western blot analysis of NF-kB p65 using anti-NF-kB p65 antibody (A00284-1). Lane 1: HepG2 whole cell lysates (30ug), Lane 2: Rabbit control IgG instead of anti-NF-kB p65 antibody in HepG2 whole cell lysate, Lane 3: anti-NF-kB p65 antibody (2ug) + HepG2 whole cell lysate (500ug). After electrophoresis, proteins were transferred to a membrane. Then the membrane was incubated with rabbit anti-NF-kB p65 antigen affinity purified polyclonal antibody (A00284-1) at a dilution of 0.5 ug/mL and probed with a goat anti-rabbit IgG-HRP secondary antibody (Catalog # BA1054). The signal is developed using ECL Plus Western Blotting Substrate (Catalog # AR1196-200). A specific band was detected for NF-kB p65 at approximately 65-70 kDa. The expected band size for NF-kB p65 is at 60 kDa.

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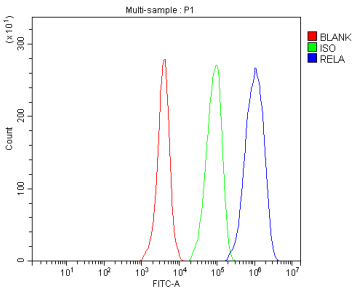
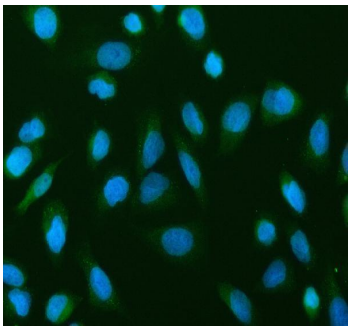
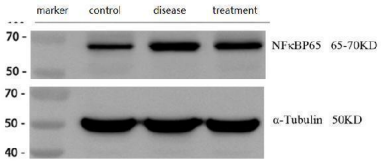


Flow Cytometry analysis of Hela cells using anti-NF-kB p65 antibody (A00284-1). Overlay histogram showing Hela cells stained with A00284-1 (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-NF-kB p65 Antibody (A00284-1, 1 ug/1x106 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x106 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x106) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



IF analysis of NF-kB p65 using anti-NF-kB p65 antibody (A00284-1). NF-kB 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 ug/mL rabbit anti-NF-kB p65 Antibody (A00284-1) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) 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.



Western blot analysis of NF-kB p65 using anti-NF-kB p65 antibody (A00284-1). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 80V (Stacking gel) / 120V (Resolving gel) for 2 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: control group-normal mouse hippocampal tissue lysates, Lane 2: hippocampal tissue from Alzheimer's disease model mouse, Lane 3: hippocampal tissue from Alzheimer's disease model mouse treated with a self-developed drug. 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 p65antigen affinity purified polyclonal antibody (A00284-1) at 1:2000 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 (Catalog # BA1054) at a dilution of 1:10000 for 1 hour at RT. The signal is developed using an ECL Plus Western Blotting Substrate (Catalog # AR1196-200) with ChemiDoc MP system. A specific band was detected for NF-kB p65 at approximately 65-70 kDa. The expected band size for NF-kB p65 is at 70 kDa.

116 Publications Citing This Product

1. PubMed ID: 10.3389/fphar.2017.00044, Celastrol Attenuates Multiple Sclerosis and Optic Neuritis in an Experimental Autoimmune Encephalomyelitis Model

2. PubMed ID: PMID:25337218, Effects of hydroxysafflor yellow A on proliferation and collagen synthesis of rat vascular adventitial fibroblasts

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induced by angiotensin II

3. PubMed ID: 10.1620/tjem.222.7, Growth Hormone Releasing Peptide-2, a Ghrelin Agonist, Attenuates Lipopolysaccharide-Induced Acute Lung Injury in Rats

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