

Anti-Phospho-N kappa-p65 (T254) RELA Antibody

Catalog Number: A00284T254

Overview

Product Name	Anti-Phospho-N kappa-p65 (T254) RELA Antibody
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-Phospho-N kappa-p65 (T254) RELA Antibody catalog # A00284T254. Tested in WB, IHC, IF, IP, ELISA applications. This antibody reacts with Human, Mouse, Rat.
Application	ELISA, IP, IF, IHC, WB
Clonality	Polyclonal
Formulation	Liquid in PBS containing 50% glycerol, 0.5% stabilizing protein and 0.02% sodium azide. *This antibody is supplied in a stabilized formulation. Compatibility with conjugation reactions depends on the chemistry of the conjugation method used. For conjugation methods that are not compatible with the stabilizing components present in this formulation, a carrier-free antibody format is required.
Storage Instructions	Store at -20°C for one year. For short term storage and frequent use, store at 4°C for up to one month. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	Q04206

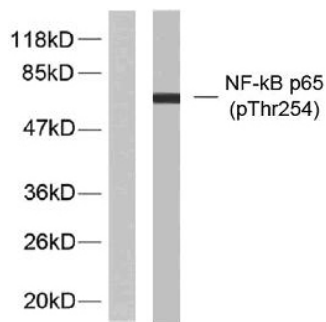
Technical Details

Immunogen	The antiserum was produced against synthesized peptide derived from human NF-kappaB p65 around the phosphorylation site of Thr254. AA range:221-270
Isotype	IgG
Form	Liquid
Concentration	1 mg/ml
Purification	The antibody was affinity-purified from rabbit antiserum by affinity-chromatography using epitope-specific immunogen.
Suggested Dilutions	WB 1:500-1:2000 IHC 1:100-1:300 IP 2-5 ug/mg lysate ELISA 1:20000 IF 1:50-200

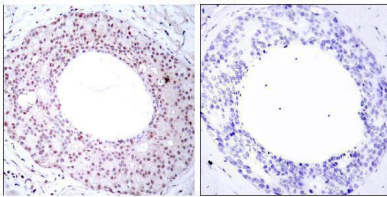
Anti-Phospho-N kappa-p65 (T254) RELA Antibody (A00284T254) Images



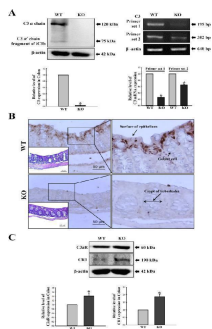
Western Blot analysis of various cells using Phospho-NFkappaB-p65 (T254) Polyclonal Antibody



Western blot analysis of lysates from 293 cells treated with TNF-alpha, using NF-kappaB p65 (Phospho-Thr254) Antibody. The lane on the left is blocked with the phospho peptide.

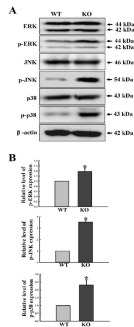


Immunohistochemistry analysis of paraffin-embedded human breast carcinoma, using NF-kappaB p65 (Phospho-Thr254) Antibody. The picture on the right is blocked with the phospho peptide.

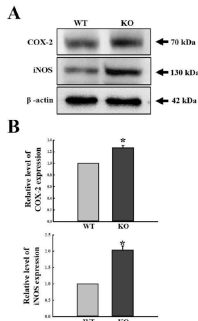


Expression levels of C3 protein and mRNA in the mid colon of C3 KO mice. (A) The expressions of C3 protein and mRNA in the mid colon were measured by applying Western blot and RT-PCR analysis, using anti-C3 antibody and C3 specific primers. After determining the intensity of each band using an imaging densitometer, relative levels of the C3 protein were calculated, based on the intensity of beta-actin. The mRNA level of the C3 gene was calculated based on the intensity of beta-actin as an endogenous control. Tissue samples were collected from 3 to 5 mice per group, and each lysate was analyzed in duplicate for Western blot and RT-PCR analysis (final n = 6-10). (B) Tissue distribution of C3 protein was analyzed in the mid colon of WT and C3 KO mice. The C3 protein-specific antibody-stained sections of the mid colon from the WT and KO mice were observed at 400 \times magnification using light microscopy. The large image in the right column is a magnified image of the rectangle in the left column. H&E-stained sections (low rectangle in left corner) were observed at 400 \times magnification using a light microscope. (C) The expressions of C3aR and CR1 protein in the mid colon were measured with Western blot analysis using anti-C3aR and CR1 antibodies. After determining the intensity of each band using an imaging densitometer,

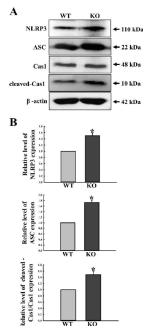
relative levels of C3aR and CR1 proteins were calculated, based on the intensity of beta-actin. Tissue samples were collected from 3 to 5 mice per group, and each lysate was analyzed in duplicate for Western blot (final n = 6-10). Data are reported as the mean \pm SD. * indicates p



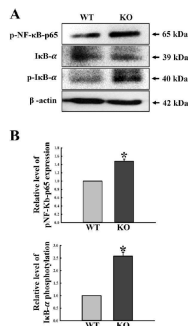
Expression levels of MAPK signaling pathway components. (A) Expression levels of ERK, p-ERK, JNK, p-JNK, p38 and p-p38 proteins were determined by Western blot analysis using the specific primary antibody and HRP-labeled anti-rabbit IgG antibody. (B) Band intensities were determined using an imaging densitometer, and protein expressions were calculated relative to the intensity of beta-actin. Tissue samples were collected from 3 to 5 mice per group, and each lysate was analyzed in duplicate for Western blot (final n = 6-10). Data are reported as the mean \pm SD. * indicates p



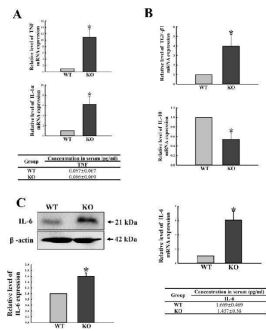
Expression levels of members in the iNOS-mediated COX-2 induction pathway. (A) Expression levels of COX-2 and iNOS proteins were determined by Western blot analysis using specific primary antibody and HRP-labeled anti-rabbit IgG antibody. (B) Band intensities were determined using an imaging densitometer, and protein expressions were calculated relative to the intensity of beta-actin. Tissue samples were collected from 3 to 5 mice per group, and each lysate was analyzed in duplicate for Western blot (final n = 6-10). Data are reported as the mean \pm SD. * indicates p



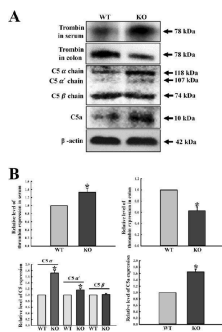
Expression levels of members in the ASC-inflammasome pathway. (A) Expression levels of NLRP3, cleaved-Cas1/Cas1 and ASC proteins were determined by Western blot analysis using the specific primary antibody and HRP-labeled anti-rabbit IgG antibody. (B) Band intensities were determined using an imaging densitometer, and protein expressions were calculated relative to the intensity of beta-actin. Tissue samples were collected from 3 to 5 mice per group, and each lysate was analyzed in duplicate for Western blot (final n = 6-10). Data are reported as the mean \pm SD. * indicates p



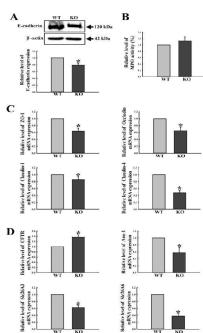
Expression levels of members in the NF-kappaB signaling pathway. (A) Expression levels of NF-kappaB-p65 and I kappaB-alpha proteins were determined by Western blot analysis using specific primary antibody and HRP-labeled anti-rabbit IgG antibody. (B) Band intensities were determined using an imaging densitometer, and protein expressions were calculated relative to the intensity of beta-actin. Tissue samples were collected from 3 to 5 mice per group, and each lysate was analyzed in duplicate for Western blot (final n = 6-10). Data are reported as the mean \pm SD. * indicates p



Levels of pro-inflammatory and anti-inflammatory cytokines. (A) The levels of TNF and IL-1α transcripts in the total mRNA of colon tissue were measured by qRT-PCR analyses using sense and anti-sense primers set for TNF and IL-1α. Concentration of the TNF protein was measured in the serum of WT and C3 KO mice using the ELISA Kit. This assay detects concentrations as low as 3.5 pg/mL for TNF. (B) The levels of anti-inflammatory cytokines including TGF-β1 and IL-10 transcripts in the total mRNA of colon tissue were measured by qRT-PCR analyses using the sense and anti-sense primer set for TGF-β1 and IL-10. The mRNA level of each gene was calculated based on the intensity of actin as an endogenous control. Tissue samples were collected from 3 to 5 mice per group, and each lysate was analyzed in duplicate for qRT-PCR (final n = 6-10). (C) Expression levels of IL-6 protein were determined by Western blot analysis using specific primary antibody and HRP-labeled anti-rabbit IgG antibody. Band intensities were determined using an imaging densitometer, and protein expressions were calculated relative to the intensity of actin. Tissue samples were collected from 3 to 5 mice per group, and each lysate was analyzed in duplicate for ELISA (final n = 6-10). The concentrations of IL-6 proteins were measured in the serum of WT and C3 KO mice using the ELISA Kit. This assay detects concentrations as low as 2 pg/mL for IL-6. Data are reported as the mean ± SD. * indicates p



Levels of C5 and its mediated inflammatory regulators. (A) Expression levels of thrombin and C5 proteins were determined by Western blot analysis using specific primary antibody and HRP-labeled anti-rabbit IgG antibody. (B) Band intensities were determined using an imaging densitometer, and protein expressions were calculated relative to the intensity of beta-actin. Tissue samples were collected from 3 to 5 mice per group, and each lysate was analyzed in duplicate for Western blot (final n = 6-10). Data are reported as the mean ± SD. * indicates p



Alterations in neutrophil infiltration and leaky epithelium. (A) Expression levels of E-cadherin were determined by Western blot analysis using specific primary antibody and HRP-labeled anti-rabbit IgG antibody. Band intensities were determined using an imaging densitometer, and protein expressions were calculated relative to the intensity of beta-actin. Tissue samples were collected from 3 to 5 mice per group, and each lysate was analyzed in duplicate for Western blot (final n = 6-10). (B) MPO activity for neutrophil level. MPO activity was measured in lysate of mid colon tissues using the MPO assay kit. Tissue samples were collected from 3 to 5 mice per group, and each lysate was analyzed in duplicate for ELISA (final n = 6-10). (C) The levels of four tight junction channels including ZO-1, Occludin, Claudin-1 and Claudin-4 transcripts in the total mRNA of colon tissue were measured by qRT-PCR analyses using sense and anti-sense primers set for ZO-1, Occludin, Claudin-1 and Claudin-4. (D) The levels of four tight junction channels including CFTR, Ano-1, Slc26A3 and Slc26A6 transcripts in the total mRNA of colon tissue were

measured by qRT-PCR analyses using sense and anti-sense primers set for CFTR, Ano-1, Slc26A3 and Slc26A6. The mRNA level of each gene was calculated based on the intensity of actin as an endogenous control. Tissue samples were collected from 3 to 5 mice per group, and each lysate was analyzed in duplicate for qRT-PCR (final n = 6-10). Data are reported as the mean \pm SD. * indicates p

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