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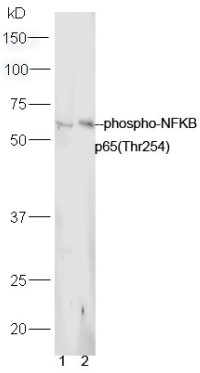
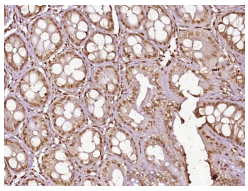
phospho-NFKB p65 (Thr254) Rabbit pAb

Catalog Number:	bs-5662R
Target Protein:	phospho-NFKB p65 (Thr254)
Concentration:	1mg/ml
Form:	Liquid
Host:	Rabbit
Clonality:	Polyclonal
Isotype:	IgG
Applications:	WB (1:500-2000), IHC-P (1:100-500), IHC-F (1:100-500), IF (1:100-500), Flow-Cyt (2ug/Test)
Reactivity:	Human, Mouse, Rat (predicted:Pig, Cow, Dog)
Predicted MW:	61 kDa
Entrez Gene:	5970
Swiss Prot:	Q04206
Source:	KLH conjugated Synthesised phosphopeptide derived from human NFKBp65 around the phosphorylation site of Thr254: FR(p-T)PP.
Purification:	affinity purified by Protein A
Storage:	0.01M TBS (pH7.4) with 1% BSA, 0.02% Proclin300 and 50% Glycerol. Shipped at 4°C. Store at -20°C for one year. Avoid repeated freeze/thaw cycles.
Background:	NF-kappa-B is a ubiquitous transcription factor involved in several biological processes. It is held in the cytoplasm in an inactive state by specific inhibitors. Upon degradation of the inhibitor, NF-kappa-B moves to the nucleus and activates transcription of specific genes. NF-kappa-B is composed of NFKB1 or NFKB2 bound to either REL, RELA, or RELB. The most abundant form of NF-kappa-B is NFKB1 complexed with the product of this gene, RELA. Four transcript variants encoding different isoforms have been found for this gene. [provided by RefSeq, Sep 2011].

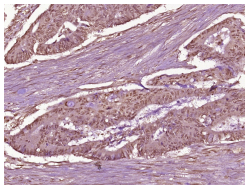
VALIDATION IMAGES



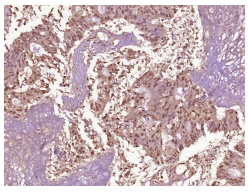
Sample: Lung (Mouse) Lysate at 40 ug Spleen (Mouse) Lysate at 40 ug Primary: Anti- phospho-NFKB p65(Thr254) (bs-5662R) at 1/300 dilution Secondary: HRP conjugated Goat-Anti-rabbit IgG (bs-0295G-HRP) at 1/5000 dilution Predicted band size: 61 kD Observed band size: 61 kD



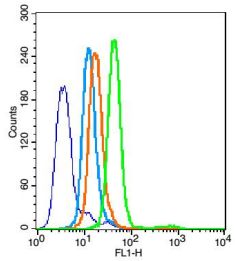
Paraformaldehyde-fixed, paraffin embedded (Rat colon); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (phospho-NFKB p65(Thr254)) Polyclonal Antibody, Unconjugated (bs-5662R) at 1:400 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining.



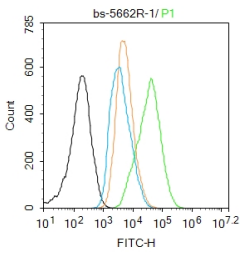
Paraformaldehyde-fixed, paraffin embedded (Human cervical carcinoma); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (phospho-NFKB p65(Thr254)) Polyclonal Antibody, Unconjugated (bs-5662R) at 1:400 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining.



Paraformaldehyde-fixed, paraffin embedded (Human stomach carcinoma); Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15min; Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes; Blocking buffer (normal goat serum) at 37°C for 30min; Antibody incubation with (phospho-NFKB p65(Thr254)) Polyclonal Antibody, Unconjugated (bs-5662R) at 1:400 overnight at 4°C, followed by operating according to SP Kit(Rabbit) (sp-0023) instructionsand DAB staining.



Overlay histogram showing HL 60 cells stained with bs-5662R (Green line). The cells were fixed with 90% methanol (5 min) and then permeabilized with 0.01M PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (bs-5662R,3μg/1x10^6 cells) for 30 min at 22°C. The secondary antibody used was fluorescein isothiocyanate goat anti-rabbit IgG (H+L) (bs- 0295G-FITC , Brillant blue line) at 1/200 dilution for 30 min at 22°C. Isotype control antibody was rabbit IgG (polyclonal,bs-0295P,Orange line) (3μg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of 20,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Blank control:A431. Primary Antibody (green line): Rabbit Anti-phospho-NFKB p65 (Thr254) antibody (bs-5662R) Dilution: 1μg /10^6 cells; Isotype Control Antibody (orange line): Rabbit IgG . Secondary Antibody : Goat anti-rabbit IgG-FITC Dilution: 1μg /test. Protocol The cells were fixed with 4% PFA (10min at room temperature)and then permeabilized with 90% ice-cold methanol for 20 min at-20°C. The cells were then incubated in 5%BSA to block non-specific protein-protein interactions for 30 min at room temperature .Cells stained with Primary Antibody for 30 min at room temperature. The secondary antibody used for 40 min at room temperature. Acquisition of 20,000 events was performed.

PRODUCT SPECIFIC PUBLICATIONS

[IF=6.1] Peiyi Wang. et al. Phenolics from Dendrobium officinale Leaf Ameliorate Dextran Sulfate Sodium-Induced Chronic Colitis by Regulating Gut Microbiota and Intestinal Barrier. J AGR FOOD CHEM. 2023;XXXX(XXX):XXX-XXX WB ; Mouse . 37883687

[IF=5.6] Keyi Nong. et al. Effect of the Pseudopleuronectes americanus-derived Pleurocidin on DSS-induced Ulcerative colitis in mice and its preliminary molecular mechanisms. INT IMMUNOPHARMACOL. 2024 Mar;130:111757 WB ; Mouse . 38422770

[IF=5.162] Lei Zhao. et al. Proteomic analysis reveals the molecular mechanism of Hippophae rhamnoides polysaccharide intervention in LPS-induced inflammation of IPEC-J2 cells in piglets. Int J Biol Macromol. 2020 Dec;164:3294 WB ; Pig . 32888998

[IF=5.4] Feiyan Tao. et al. Inhibition of p38 MAPK/NF-κB p65 signaling pathway activity by rare ginsenosides ameliorates cyclophosphamide-induced premature ovarian failure and KGN cell injury. J ETHNOPHARMACOL. 2024 May;326:117944 WB ; Rat,Human . 38382656

[IF=4.6] Han Juanjuan. et al. Moderate mechanical stress suppresses chondrocyte ferroptosis in osteoarthritis by regulating NF-κB p65/GPX4 signaling pathway. SCI REP-UK. 2024 Mar;14(1):1-15 IHC ; Rat . 38429394