

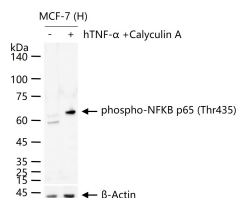
bs-5661R**[Primary Antibody]****phospho-NFKB p65 (Thr435) Rabbit pAb****Bioss**
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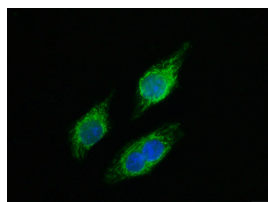
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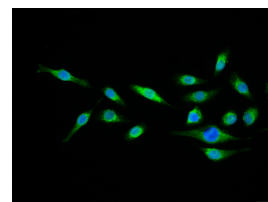
400-901-9800

DATASHEET**Host:** Rabbit**Isotype:** IgG**Clonality:** Polyclonal**GeneID:** 5970**SWISS:** Q04206**Target:** NFKB p65 (Thr435)**Immunogen:** KLH conjugated Synthesised phosphopeptide derived from human NFKBp65 around the phosphorylation site of Thr435: EG(p-T)LS.**Purification:** affinity purified by Protein A**Concentration:** 1mg/ml**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].**Applications:** WB (1:500-2000)**Flow-Cyt** (2ug/Test)**ICC/IF** (1:100-500)**Reactivity:** Human, Mouse
(predicted: Rat)**Predicted
MW.:** 61 kDa**Observed
MW.:** 65 kDa**Subcellular
Location:** Cytoplasm ,Nucleus**VALIDATION IMAGES**

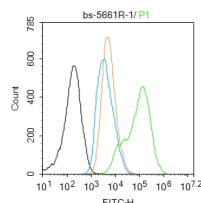
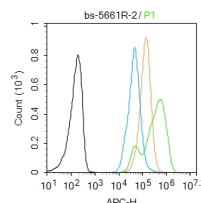
MCF-7 (H) cells were treated with or without hTNF-α (20 ng/ml) and Calyculin A (100 nM) for 15 min, 25 µg total protein per lane of cell lysates (see on figure) probed with phospho-NFKB p65 (Thr435) polyclonal antibody, unconjugated (bs-5661R) at 1:1000 dilution and 4°C overnight incubation. Followed by conjugated secondary antibody incubation at r.t. for 60 min.



Tissue/cell: MCF7 cell; 4% Paraformaldehyde-fixed; Triton X-100 at room temperature for 20 min; Blocking buffer (normal goat serum, C-0005) at 37°C for 20 min; Antibody incubation with (phospho-NFKB p65 (Thr435)) polyclonal Antibody, Unconjugated (bs-5661R) 1:100, 90 minutes at 37°C; followed by a FITC conjugated Goat Anti-Rabbit IgG antibody at 37°C for 90 minutes, DAPI (blue, C02-04002) was used to stain the cell nuclei.



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Blank control: A431. Primary Antibody (green line): Rabbit Anti-phospho-NFKB p65 (Thr435) antibody (bs-5661R) Dilution: 1 µg / 10⁶ cells; Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody: Goat anti-rabbit IgG-FITC Dilution: 1 µg / test. Protocol The cells wereBlank control: MCF7. Primary Antibody (green line): Rabbit Anti-phospho-NFKB p65 (Thr435) antibody (bs-5661R) Dilution: 2 µg / 10⁶ cells; Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody: Goat anti-rabbit IgG-BF647 Dilution: 1 µg / test. Protocol The cells were

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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.

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— SELECTED CITATIONS —

- **[IF=9.429]** Zhu Ting-ting. et al. Melanin-like polydopamine nanoparticles mediating anti-inflammatory and rescuing synaptic loss for inflammatory depression therapy. J NANOBIOTECHNOL. 2023 Dec;21(1):1-19 WB ;Mouse. 36765377
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