

## Product Datasheet

# Phospho-NFKB p65 (Ser281) Rabbit Polyclonal Antibody (orb185656)

<b>Catalog Number</b>	orb185656
<b>Category</b>	Antibodies
<b>Description</b>	Phospho-NFKB p65 (Ser281) Rabbit Polyclonal Antibody
<b>Target</b>	RELA
<b>Clonality</b>	Polyclonal
<b>Species/Host</b>	Rabbit
<b>Isotype</b>	IgG
<b>Conjugation</b>	Unconjugated
<b>Reactivity</b>	Human, Mouse
<b>Predicted Reactivity</b>	Bovine, Canine, Equine, Porcine, Rat, Sheep
<b>Form/Appearance</b>	Liquid
<b>Concentration</b>	1mg/ml
<b>Buffer/Preservatives</b>	0.01M TBS (pH7.4) with 1% rAlbumin, 0.02% Proclin300 and 50% Glycerol.
<b>Purification</b>	Affinity purified by Protein A
<b>Immunogen</b>	KLH conjugated synthesised phosphopeptide derived from human NFKB p65 around the phosphorylation site of Ser281 EL(p-S)EP
<b>UniProt ID</b>	<b>Q04206</b>

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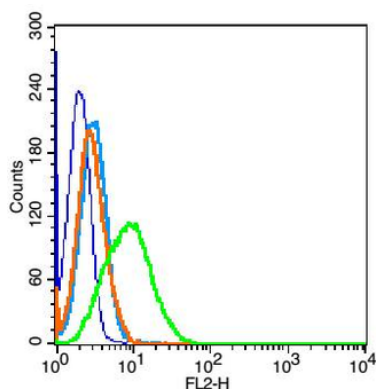
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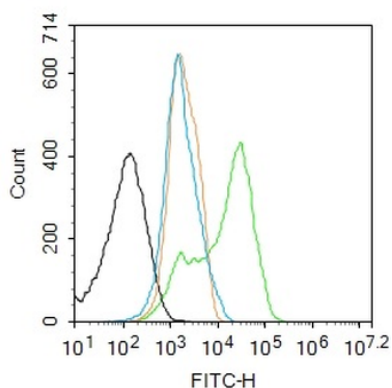
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<b>MW</b>	61 kDa
<b>Tested applications</b>	FC, WB
<b>Dilution range</b>	WB=1:500-2000, Flow-Cyt=1µg/Test
<b>Antibody Type</b>	Primary Antibody
<b>Storage</b>	Maintain refrigerated at 2-8°C for up to 2 weeks. For long term storage store at -20°C in small aliquots to prevent freeze-thaw cycles.
<b>Note</b>	For research use only
<b>Expiration Date</b>	12 months from date of receipt.



Blank control (blue): Jurkat cells (fixed with 2% paraformaldehyde (10 min), then permeabilized with 90% ice-cold methanol for 30 min on ice). Primary Antibody: Rabbit Anti-phospho-NFKB p65 (Ser281) antibody (orb185656), dilution: 1 µg in 100 µl 1X PBS containing 0.5% BSA, Isotype Control Antibody: Rabbit IgG (orange), used under the same conditions, Secondary Antibody: Goat anti-rabbit IgG-PE (white blue), dilution: 1:200 in 1X PBS containing 0.5% BSA.



Blank control: HL-60. Primary Antibody (green line): Rabbit Anti-phospho-NFKB p65 (Ser281) antibody (orb185656), dilution: 1 µg/10<sup>6</sup> cells, Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody: Goat anti-rabbit IgG-FITC, dilution: 1 µg/Test. Protocol, The cells were fixed with 4% PFA (10 min 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 20000 events was performed.

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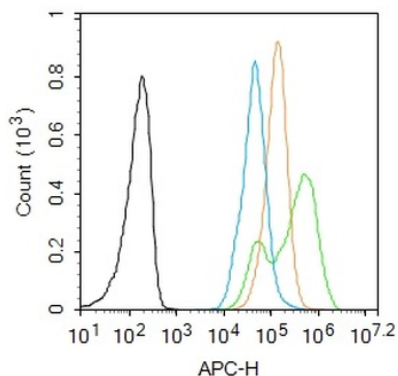
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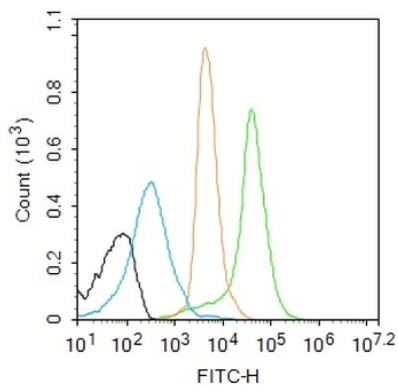
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Blank control: MCF7. Primary Antibody (green line): Rabbit Anti-phospho-NFKB p65 (Ser281) antibody (orb185656), dilution: 2  $\mu\text{g}/10^6$  cells, Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody: Goat anti-rabbit IgG-AF647, dilution: 1  $\mu\text{g}/\text{Test}$ . Protocol, The cells were fixed with 4% PFA (10 min at room temperature) and then permeabilized with 90% ice-cold methanol for 20 min at  $-20^\circ\text{C}$ . 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 20000 events was performed.



Blank control: Mouse spleen. Primary Antibody (green line): Rabbit Anti-phospho-NFKB p65 (Ser281) antibody (orb185656), dilution: 2  $\mu\text{g}/10^6$  cells, Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody: Goat anti-rabbit IgG-AF488, dilution: 1  $\mu\text{g}/\text{Test}$ . Protocol, The cells were fixed with 4% PFA (10 min at room temperature) and then permeabilized with 90% ice-cold methanol for 20 min at  $-20^\circ\text{C}$ . 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 20000 events was performed.

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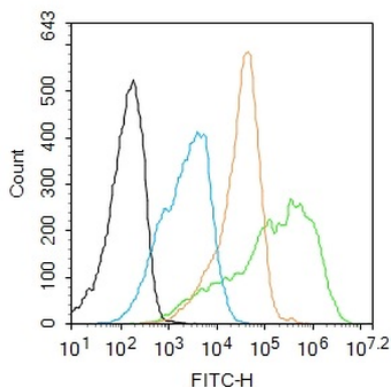
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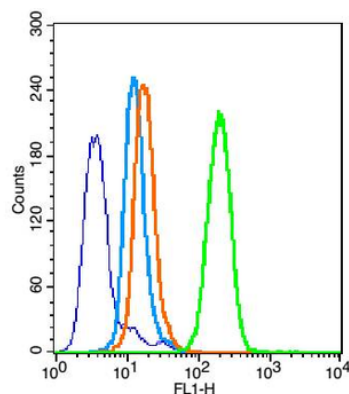
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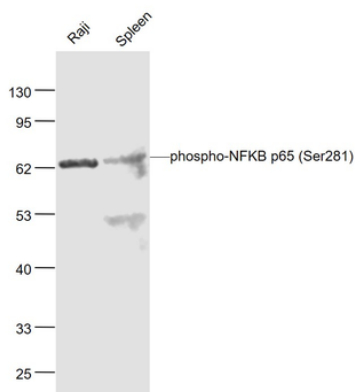
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Blank control: Mouse thymus. Primary Antibody (green line): Rabbit Anti-phospho-NFKB p65 (Ser281) antibody (orb185656), dilution: 2 µg/10<sup>6</sup> cells, Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody: Goat anti-rabbit IgG-AF488, dilution: 1 µg/Test. Protocol, The cells were fixed with 4% PFA (10 min 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 20000 events was performed.



Overlay histogram showing HL 60 cells stained with orb185656 (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 (orb185656, 1 µg/1x10<sup>6</sup> cells) for 30 min at 22°C. The secondary antibody used was fluorescein isothiocyanate goat anti-rabbit IgG (H+L) (Brilliant blue line) at 1/200 dilution for 30 min at 22°C. Isotype control antibody was rabbit IgG (polyclonal, Orange line) (1 µg/1x10<sup>6</sup> cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of 20000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



Sample: Raji (Human) Cell Lysate at 30 ug, Spleen (Mouse) Lysate at 40 ug, Primary: Anti-phospho-NFKB p65 (Ser281) (orb185656) at 1/1000 dilution, Secondary: IRDye800CW Goat Anti-Rabbit IgG at 1/20000 dilution, Predicted band size: 65 kD, Observed band size: 65 kD.

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