

## Product Datasheet

# Phospho-NFkB p65 (Ser276) Rabbit Polyclonal Antibody (orb106211)

<b>Catalog Number</b>	orb106211
<b>Category</b>	Antibodies
<b>Description</b>	Phospho-NFkB p65 (Ser276) 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, Rat
<b>Predicted Reactivity</b>	Bovine, Canine, Equine, Mouse, Porcine
<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 NFkBp65 around the phosphorylation site of Ser276 RP(p-S)DR
<b>UniProt ID</b>	<b>Q04206</b>

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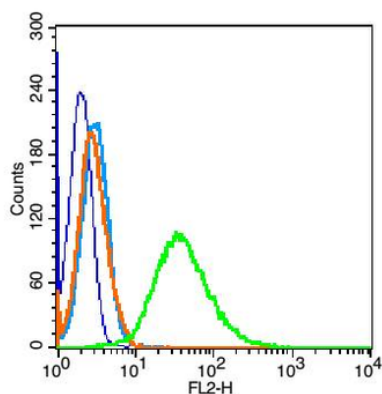
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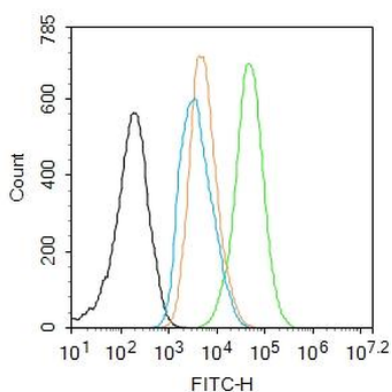
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<b>MW</b>	61 kDa
<b>Tested applications</b>	FC, ICC, IF, IHC-Fr, IHC-P
<b>Dilution range</b>	IHC-P=1:100-500, IHC-F=1:100-500, ICC/IF=1:100-500, IF=1:100-500, Flow-Cyt=0.2µg /Test
<b>Antibody Type</b>	Primary Antibody
<b>Storage</b>	Maintain refrigerated 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-NFkB p55 (Ser276)antibody antibody (orb106211), Dilution: 0.2 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 1 X PBS containing 0.5% BSA.



Blank control: A431. Primary Antibody (green line): Rabbit Anti-Phospho-NFkB p55 (Ser276) antibody (orb106211), 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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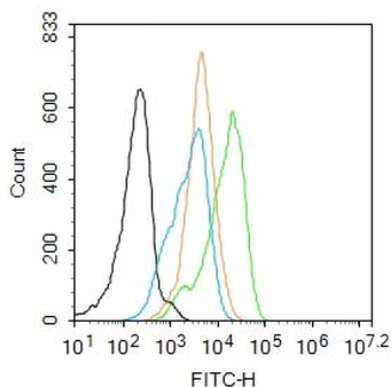
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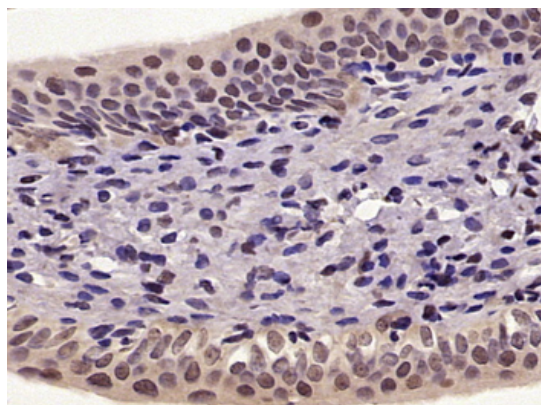
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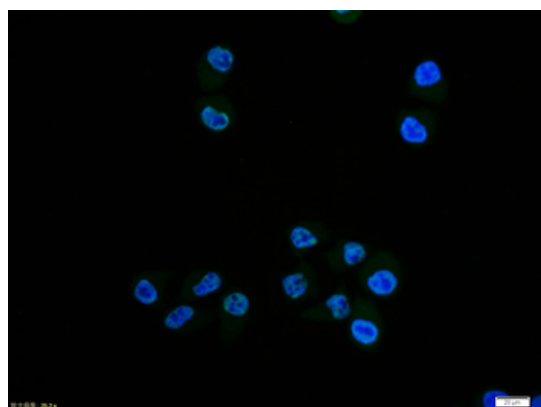
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Blank control: HL-60. Primary Antibody (green line): Rabbit Anti-Phospho-NFKB p65 (Ser276) antibody (orb106211), Dilution: 2  $\mu\text{g}/10^6$  cells, Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody: Goat anti-rabbit IgG-FITC, 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.



Paraformaldehyde-fixed, paraffin embedded (Rat urinary bladder), Antigen retrieval by microwave in sodium citrate buffer (pH6.0), Block endogenous peroxidase by 3% hydrogen peroxide for 30 minutes, Blocking buffer (3% BSA) at RT for 30 min, Antibody incubation with (Phospho-NFKB p65 (Ser276)) Polyclonal Antibody, Unconjugated (orb106211) at 1:400 overnight at  $4^\circ\text{C}$ , followed by conjugation to the secondary antibody (labeled with HRP) and DAB staining.



Tissue/Cell: HeLa cell, 4% Paraformaldehyde-fixed, Triton X-100 at room temperature for 20 min, Blocking buffer (normal goat serum) at  $37^\circ\text{C}$  for 20 min, Antibody incubation with (Phospho-NFKB p65 (Ser276)) polyclonal Antibody, Unconjugated (orb106211) 1:100, 90 minutes at  $37^\circ\text{C}$ , followed by a FITC conjugated Goat Anti-Rabbit IgG antibody at  $37^\circ\text{C}$  for 90 minutes, DAPI (blue) was used to stain the cell nuclei.

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Tissue/Cell: MCF7 cell, 4% Paraformaldehyde-fixed, Triton X-100 at room temperature for 20 min, Blocking buffer (normal goat serum) at 37°C for 20 min, Antibody incubation with (Phospho-NFKB p65 (Ser276)) polyclonal Antibody, Unconjugated 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) was used to stain the cell nuclei.

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