

## Product Datasheet

### NFKB p65 Rabbit Polyclonal Antibody (orb312399)

<b>Catalog Number</b>	orb312399
<b>Category</b>	Antibodies
<b>Description</b>	NFKB p65 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, Rat
<b>Predicted Reactivity</b>	Bovine, Canine, 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 synthetic peptide derived from human NFKB p65 (201-300/551aa)
<b>UniProt ID</b>	<b>Q04206</b>
<b>MW</b>	65 kDa

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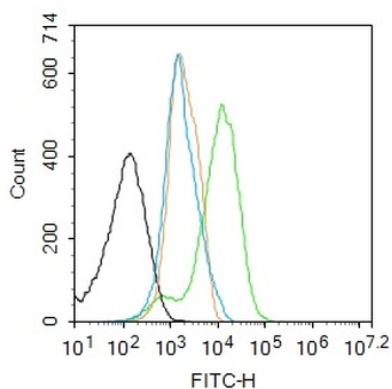
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<b>Tested applications</b>	FC, ICC, IF, IHC-Fr, IHC-P, WB
<b>Dilution range</b>	WB=1:1000-5000, IHC-P=1:100-200, IHC-F=1:100-200, ICC/IF=1:100-200, IF=1:100-200, Flow-Cyt=1:50-100
<b>Antibody Type</b>	Primary Antibody
<b>Storage</b>	Maintain refrigerated 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: HL-60. Primary Antibody (green line): Rabbit Anti-NFKB p65 antibody (orb312399), dilution: 1  $\mu\text{g}/10^6$  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°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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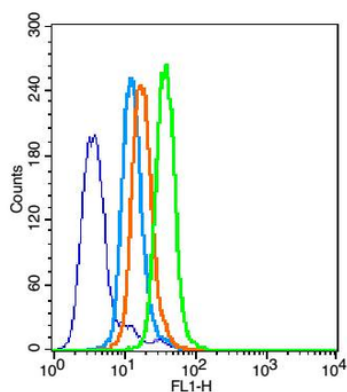
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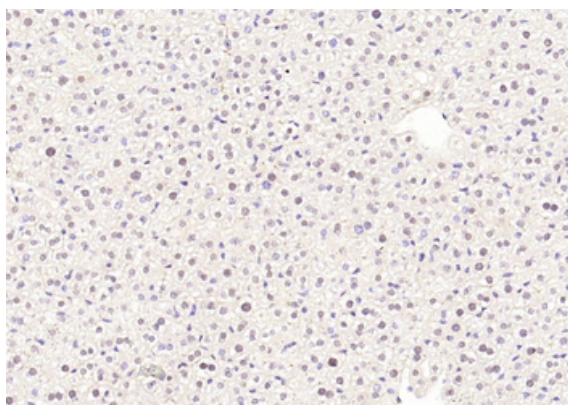
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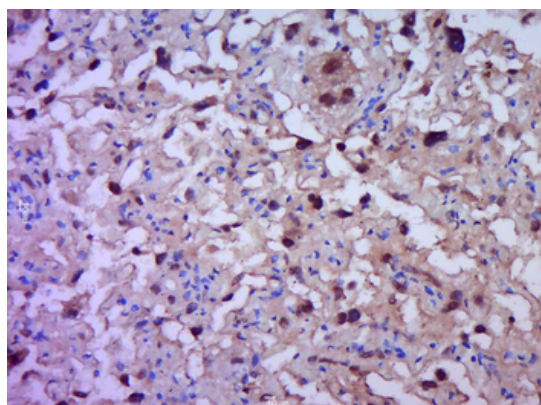
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Overlay histogram showing HL 60 cells stained with orb312399 (Green line). The cells were fixed with 90% methanol (5 min) and then permeabilized with 0.01M PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (orb312399, 3 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) (Brillant blue line) at 1/200 dilution for 30 min at 22°C. Isotype control antibody was rabbit IgG (polyclonal, Orange line) (3 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.



Paraformaldehyde-fixed, paraffin embedded (mouse liver), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (NFKB p65) Polyclonal Antibody, Unconjugated (orb312399) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (mouse placenta tissue), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (NFKB p65) Polyclonal Antibody, Unconjugated (orb312399) at 1:400 overnight at 4°C, followed by a conjugated secondary for 20 minutes and DAB staining.

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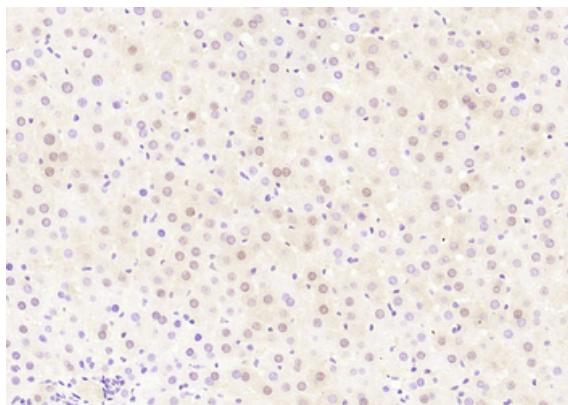
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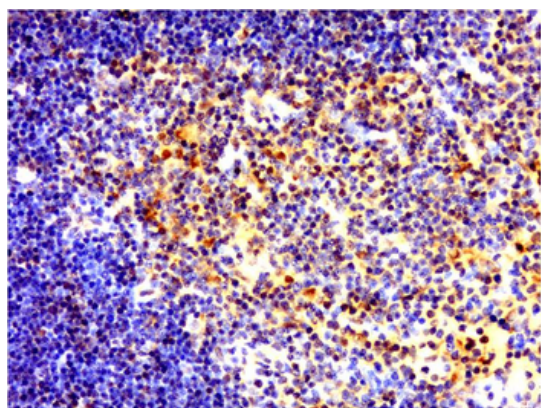
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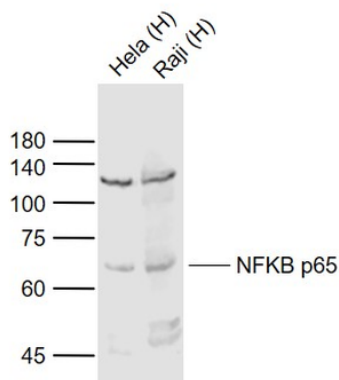
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Paraformaldehyde-fixed, paraffin embedded (rat liver), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (NFKB p65) Polyclonal Antibody, Unconjugated (orb312399) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (rat thymus), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (NFKB p65) Polyclonal Antibody, Unconjugated (orb312399) at 1:500 overnight at 4°C, followed by a conjugated secondary for 20 minutes and DAB staining.



Sample: Lane 1: HeLa (Human) Cell Lysate at 30 ug, Lane 2: Raji (Human) Cell Lysate at 30 ug, Primary: Anti-NFKB p65 (orb312399) at 1/200 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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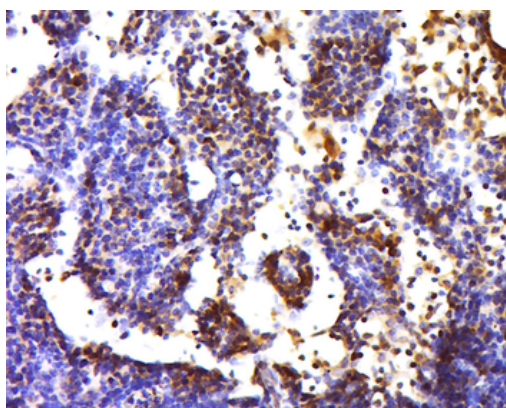
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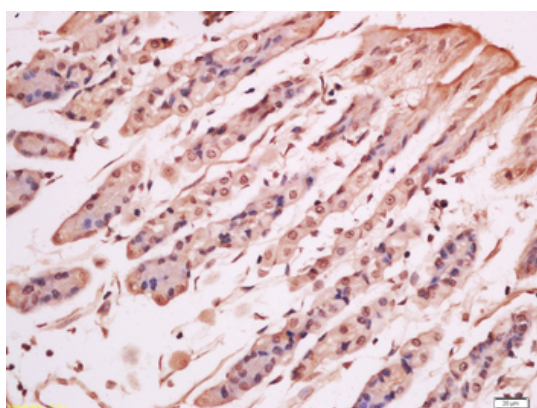
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Tissue/Cell: Mouse lymph tissue, 4% Paraformaldehyde-fixed and paraffin-embedded, Antigen retrieval: citrate buffer (0.01M, pH 6.0), Boiling bathing for 15 min, Block endogenous peroxidase by 3% Hydrogen peroxide for 30 min, Blocking buffer (normal goat serum) at 37°C for 20 min, Incubation: Anti-NFKB p65 Polyclonal Antibody, Unconjugated (orb312399) 1:500, overnight at 4°C, followed by conjugation to the secondary antibody and DAB staining.



Tissue/Cell: Mouse stomach tissue, 4% Paraformaldehyde-fixed and paraffin-embedded, Antigen retrieval: citrate buffer (0.01M, pH 6.0), Boiling bathing for 15 min, Block endogenous peroxidase by 3% Hydrogen peroxide for 30 min, Blocking buffer (normal goat serum) at 37°C for 20 min, Incubation: Anti-NFKB p65 Polyclonal Antibody, Unconjugated (orb312399) 1:500, overnight at 4°C, followed by conjugation to the secondary antibody and DAB staining.

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