

## Product Datasheet

# Phospho-FoxO1 (Ser256) Rabbit Polyclonal Antibody (orb6050)

<b>Catalog Number</b>	orb6050
<b>Category</b>	Antibodies
<b>Description</b>	Phospho-FoxO1 (Ser256) Rabbit Polyclonal Antibody
<b>Target</b>	FOXO1
<b>Clonality</b>	Polyclonal
<b>Species/Host</b>	Rabbit
<b>Isotype</b>	IgG
<b>Conjugation</b>	Unconjugated
<b>Reactivity</b>	Human, Mouse, Rabbit, Rat
<b>Predicted Reactivity</b>	Bovine, Canine, Equine, Gallus, 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 FOXO1 around the phosphorylation site of Ser256 AA(p-S)MD
<b>UniProt ID</b>	<b>Q12778</b>

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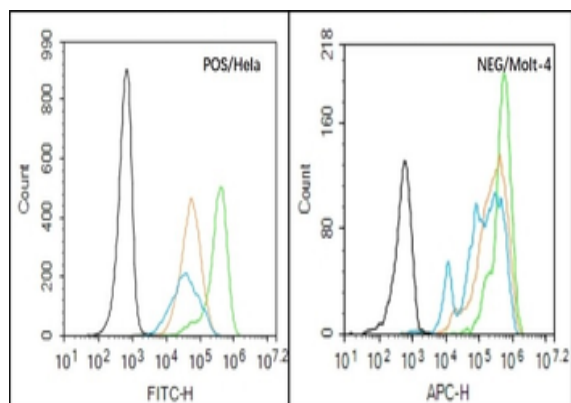
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<b>RRID</b>	AB_10933025
<b>MW</b>	72 kDa
<b>Tested applications</b>	ELISA, FC, IF, IHC-Fr, IHC-P
<b>Dilution range</b>	IHC-P=1:100-500, IHC-F=1:100-500, IF=1:100-500, Flow-Cyt=1µg /Test, ELISA=1:5000-10000
<b>Antibody Type</b>	Primary Antibody
<b>Storage</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.



Black line: Positive blank control (Hela), Negative blank control (Molt4), Green line: Primary Antibody (Rabbit Anti-Phospho-FoxO1 (Ser256) antibody (orb6050)), Orange line: Isotype Control Antibody (Rabbit IgG). Blue line: Secondary Antibody (Goat anti-rabbit IgG-AF488), Hela (Positive) and Molt4 (Negative control) cells (black) were fixed with 4% PFA for 10 min at room temperature, permeabilized with 90% ice-cold methanol for 20 min at -20°C, and incubated in 5% BSA blocking buffer for 30 min at room temperature. Cells were then stained with Phospho-FoxO1 (Ser256) Antibody (orb6050) at 1:50 dilution in blocking buffer and incubated for 30 min at room temperature, washed twice with 2% BSA in PBS, followed by secondary antibody (blue) incubation for 40 min at room temperature. Acquisitions of 20000 events were performed. Cells stained with primary antibody (green), and isotype control (orange).

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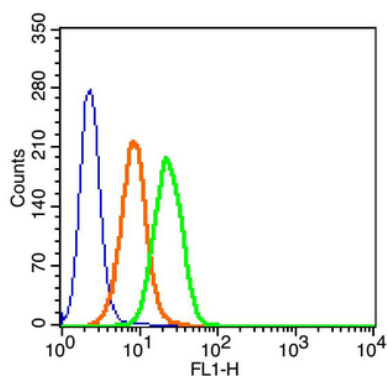
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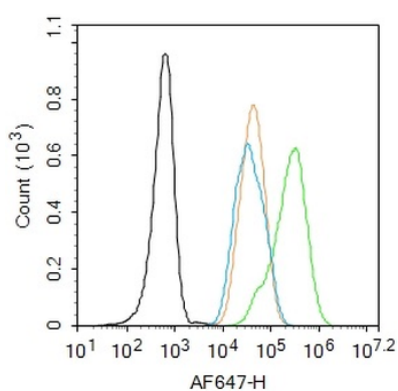
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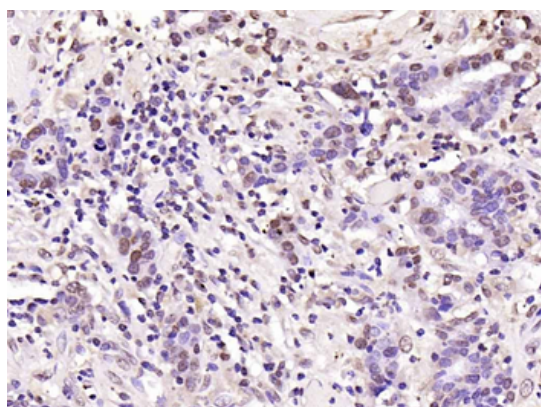
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Blank control (blue): HeLa (fixed with 2% paraformaldehyde (10 min) and then permeabilized with ice-cold 90% methanol for 30 min on ice). Primary Antibody: Rabbit Anti-Phospho-FoxO1 (Ser256)/FITC antibody, dilution: 1 µg in 100 µl 1X PBS containing 0.5% BSA, Isotype Control Antibody: Rabbit IgG/FITC (orange), used under the same conditions.



Blank control: HepG2. Primary Antibody (green line): Rabbit Anti-Phospho-FoxO1 (Ser256) antibody (orb6050), dilution: 1 µg/10<sup>6</sup> cells, Isotype Control Antibody (orange line): Rabbit IgG. Secondary Antibody: Goat anti-rabbit IgG-AF647, 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.



Paraformaldehyde-fixed, paraffin embedded (human gastric carcinoma), 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 (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (orb6050) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

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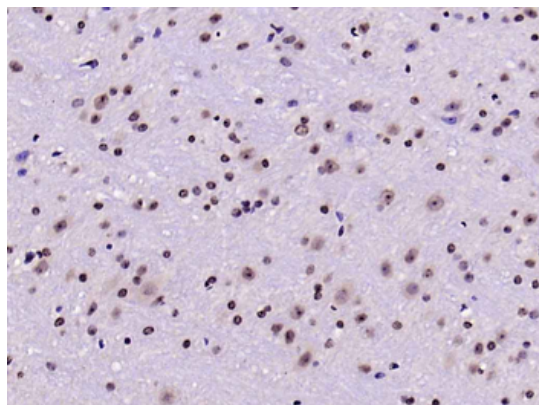
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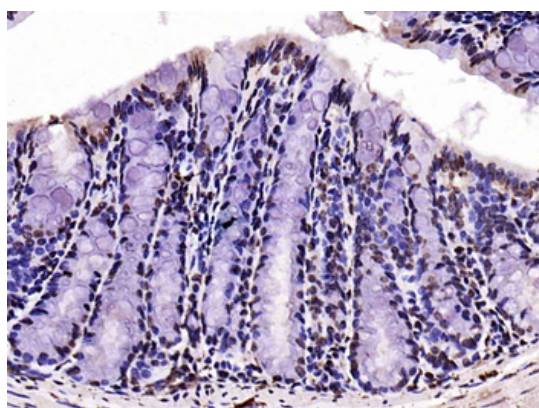
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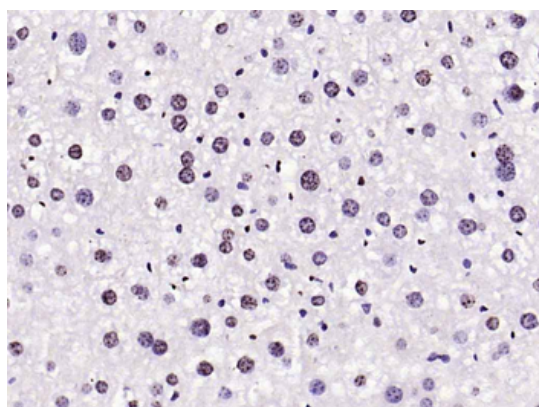
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Paraformaldehyde-fixed, paraffin embedded (mouse brain), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (orb6050) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (mouse colon), 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 (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (orb6050) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



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 (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (orb6050) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

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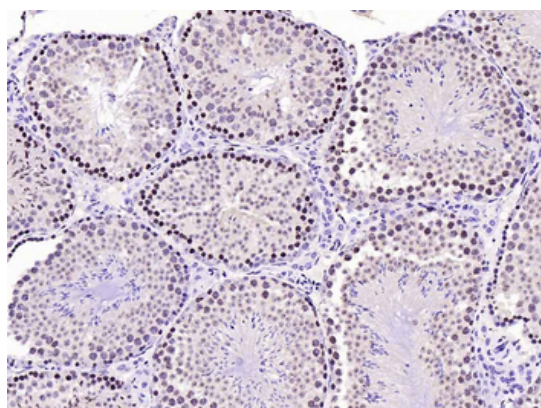
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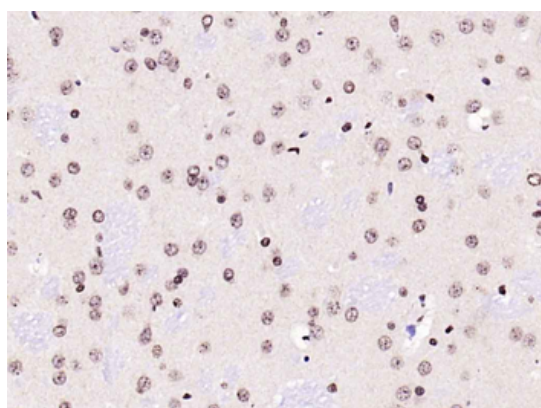
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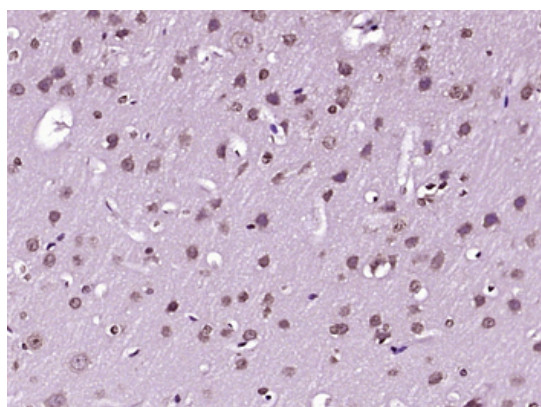
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Paraformaldehyde-fixed, paraffin embedded (mouse testis), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (orb6050) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (rat brain), 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 (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (orb6050) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (Rat brain), 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 (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (orb6050) at 1:400 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

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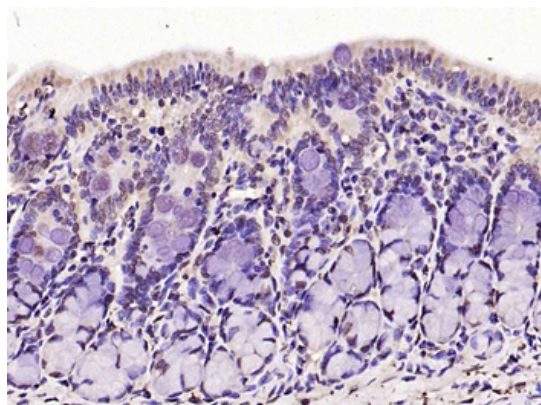
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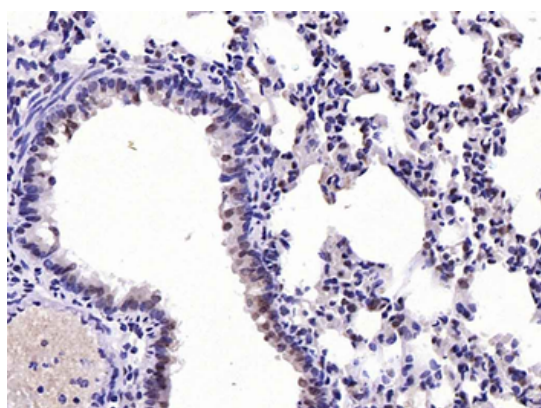
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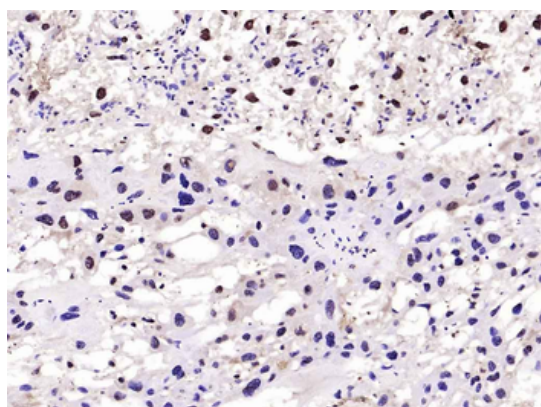
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Paraformaldehyde-fixed, paraffin embedded (rat colon), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (orb6050) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (rat lung), 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 (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (orb6050) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



Paraformaldehyde-fixed, paraffin embedded (rat placenta), 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 (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (orb6050) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

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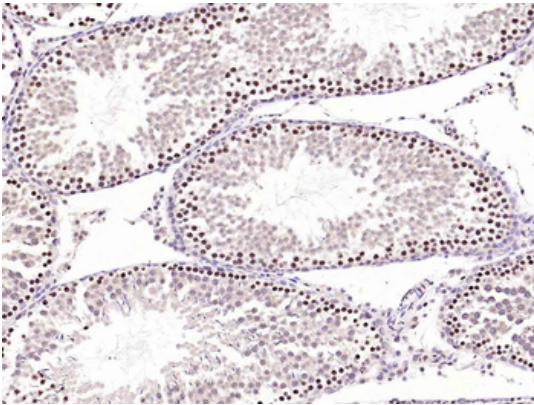
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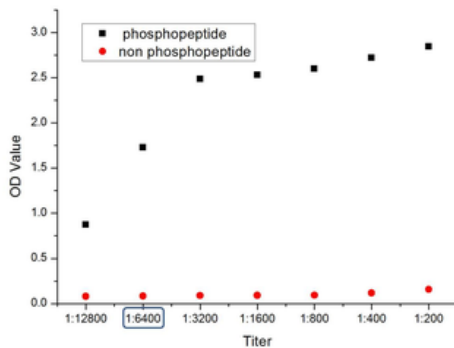
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Paraformaldehyde-fixed, paraffin embedded (rat testis), Antigen retrieval by boiling in sodium citrate buffer (pH6.0) for 15 min, Block endogenous peroxidase by 3% hydrogen peroxide for 20 minutes, Blocking buffer (normal goat serum) at 37°C for 30 min, Antibody incubation with (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (orb6050) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.



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