

## Anti-Phospho-FOXO1 (Ser256) Polyclonal Antibody

## Product Details

Ig Type:	IgG
Reactivity:	Human,Mouse,Rat,Rabbit (predicted:Chicken,Dog,Pig,Cow,Horse)
Molecular Weight:	Theoretical: 72 kDa.
Purification:	Protein A purified

## Applications

1. phosphopeptide  
non phosphopeptide
2. 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 (TMAB-01412-FITC), Dilution: 1 µg in 100 µL 1X PBS containing 0.5% BSA; Isotype Control Antibody: Rabbit IgG/FITC (orange), used under the same conditions.
3. Black line: Positive blank control (Hela); Negative blank control (Molt4)  
Green line: Primary Antibody (Rabbit Anti-Phospho-FoxO1 (Ser256) antibody (TMAB-01412))  
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 (TMAB-01412) 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 20,000 events were performed. Cells stained with primary antibody (green), and isotype control (orange).
4. 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 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (TMAB-01412) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.
5. 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 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (TMAB-01412) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.
6. 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 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (TMAB-01412) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.
7. 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

Verified Activity:

peroxide for 20 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (TMAB-01412) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

8. 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 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (TMAB-01412) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

9. 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 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (TMAB-01412) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

10. 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 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (TMAB-01412) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

11. 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 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (TMAB-01412) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

12. 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 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (TMAB-01412) at 1:200 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.

13. Blank control: HepG2. Primary Antibody (green line): Rabbit Anti-Phospho-FoxO1 (Ser256) antibody (TMAB-01412)

Dilution: 1 µg/10<sup>6</sup> cells;

Isotype Control Antibody (orange line): Rabbit IgG.

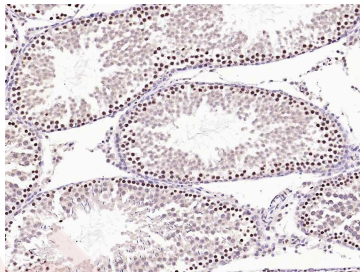
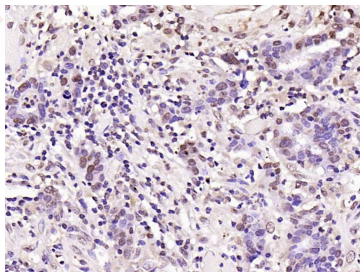
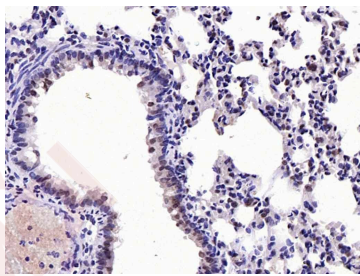
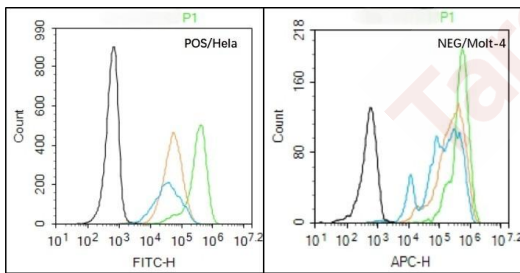
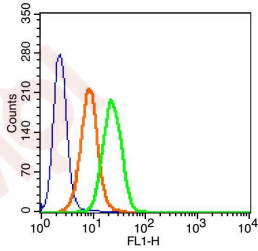
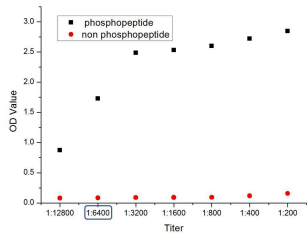
Secondary Antibody: Goat anti-rabbit IgG-AF647

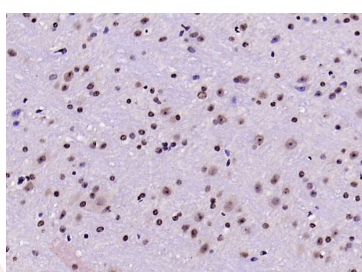
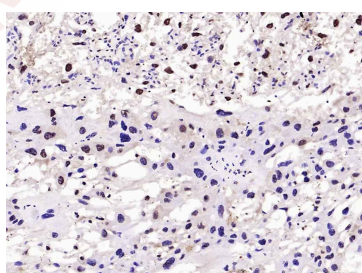
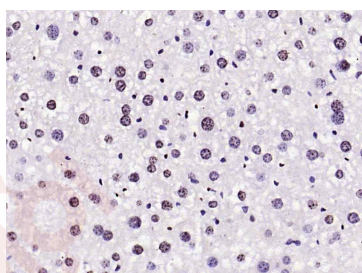
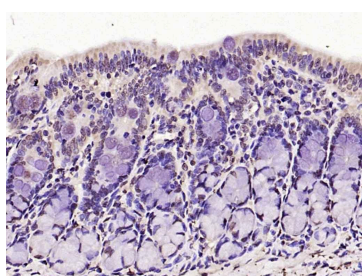
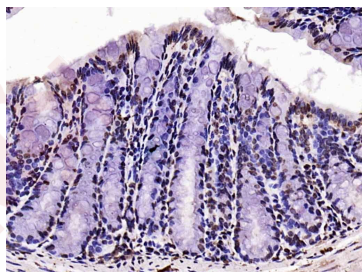
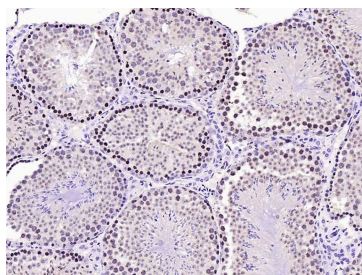
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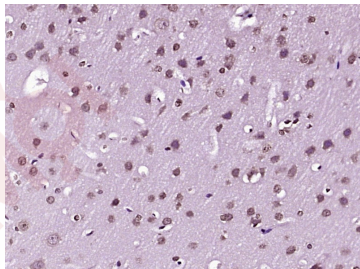
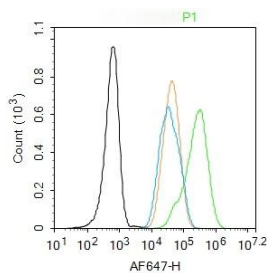
Protocol

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.

14. 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 min; Blocking buffer (normal goat serum) at 37°C for 30 min; Antibody incubation with (Phospho-FoxO1 (Ser256)) Polyclonal Antibody, Unconjugated (TMAB-01412) at 1:400 overnight at 4°C, followed by operating according to SP Kit (Rabbit) instructions and DAB staining.







Application: ELISA,FCM,IF,IHC-Fr,IHC-P

Recommended IHC-P: 1:100-500; IHC-Fr: 1:100-500; IF: 1:100-500; FCM: 1µg /Test; ELISA: 1:5000-10000

### Properties

Stability & Storage: Store at -20°C or -80°C for 12 months. Avoid repeated freeze-thaw cycles.

Shipping: Shipping with blue ice.

### Antigen Details

Immunogen: KLH conjugated Synthesised phosphopeptide: human FOXO1 around the phosphorylation site of Ser256

Antigen Species: Human

Gene ID: 2308

Uniprot ID: Q12778

Synonyms: Forkhead box protein O1;FOXO1 (p-Ser256);FOXO1A;Forkhead(Drosophila) homolog 1 (rhabdomyosarcoma);AI876417;FKH1;p-FOXO1 (Ser256);p-FOXO1 (S256);Forkhead in rhabdomyosarcoma;Forkhead box O1;Afxh;Forkhead;Forkhead box protein O1A;Fkhr1;FOXO1 (p-S256);Forkhead, Drosophila, homolog of, in rhabdomyosarcoma;FKH 1;FKHR

Biology Area: Coactivators and repressors,Co-activators/co-repressors,FOXO,Obesity,Neurogenesis,Co-activators/co-repressors

### Research Background

This gene belongs to the forkhead family of transcription factors which are characterized by a distinct forkhead domain. The specific function of this gene has not yet been determined; however, it may play a role in myogenic growth and differentiation. Translocation of this gene with PAX3 has been associated with alveolar rhabdomyosarcoma. [provided by RefSeq].

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