

Product Datasheet

Anti-FOXO3A Antibody (orb215950)

Description	Anti-FOXO3A Antibody
Species/Host	Rabbit
Reactivity	Human, Rat
Conjugation	Unconjugated
Tested Applications	FC, ICC, IF, IHC, WB
Immunogen	E.coli-derived human FOXO3A recombinant protein (Position: Q471-G673). Human FOXO3A shares 97% amino acid (aa) sequence identity with mouse FOXO3A.
Form/Appearance	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.
Storage	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.
Note	For research use only
Application notes	Western blot, 0.1-0.5µg/ml, Human, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5µg/ml, Human Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human Flow Cytometry(Fixed), 1-3 µg/1x10 ⁶ cells, Human. Add 0.2ml of distilled water will yield a concentration of 500ug/ml
Isotype	Rabbit IgG
Clonality	Polyclonal
Antibody Type	Primary Antibody
MW	80-90 kDa

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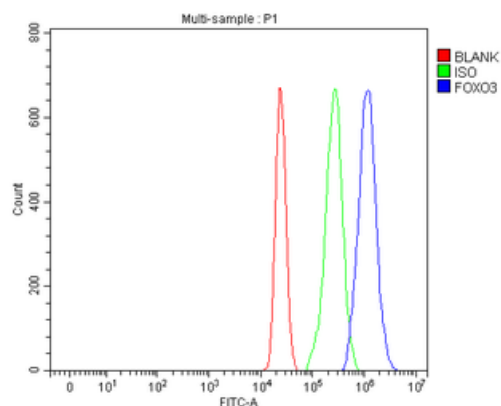
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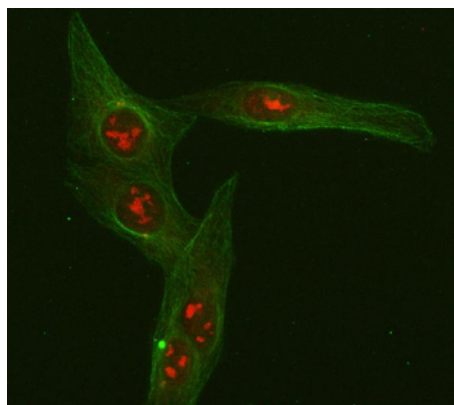
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Uniprot ID**O43524****Expiration Date**

12 months from date of receipt.



Flow Cytometry analysis of MCF-7 cells using anti-FOXO3A antibody. Overlay histogram showing MCF-7 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-FOXO3A Antibody (1 $\mu\text{g}/1 \times 10^6$ cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 $\mu\text{g}/1 \times 10^6$ cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 $\mu\text{g}/1 \times 10^6$) used under the same conditions. Unlabelled sample (Red line) was also used as a control.



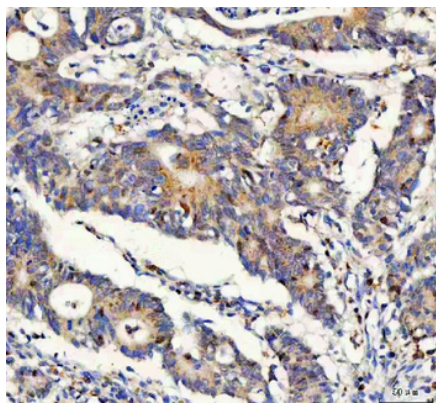
IF analysis of FOXO3A using anti-FOXO3A antibody and anti-Beta Tubulin antibody. FOXO3A was detected in immunocytochemical section of U2OS cell. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 $\mu\text{g}/\text{mL}$ rabbit anti-FOXO3A Antibody and mouse anti-Beta Tubulin antibody overnight at 4°C. Cy3 Conjugated Goat Anti-Rabbit IgG and DyLight®488 Conjugated Goat Anti-Mouse IgG were used as secondary antibody at 1:500 dilution and incubated for 30 minutes at 37°C. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

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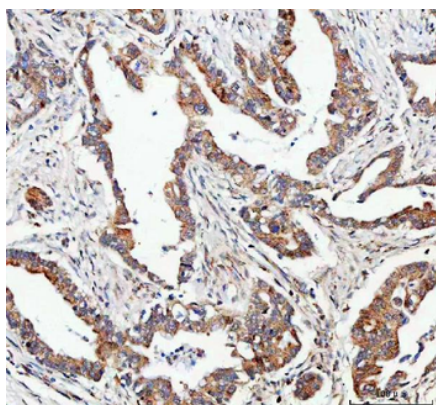
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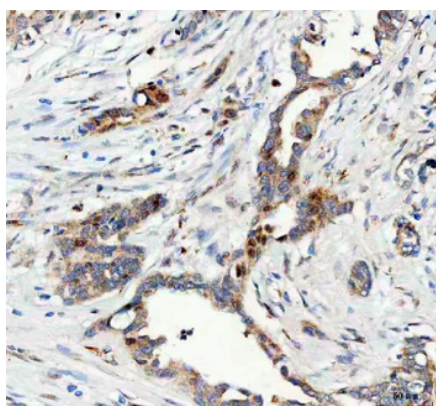
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IHC analysis of FOXO3A using anti-FOXO3A antibody. FOXO3A was detected in a paraffin-embedded section of human colorectal adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 μ g/ml rabbit anti-FOXO3A Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.



IHC analysis of FOXO3A using anti-FOXO3A antibody. FOXO3A was detected in a paraffin-embedded section of human pancreatic ductal adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 μ g/ml rabbit anti-FOXO3A Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.



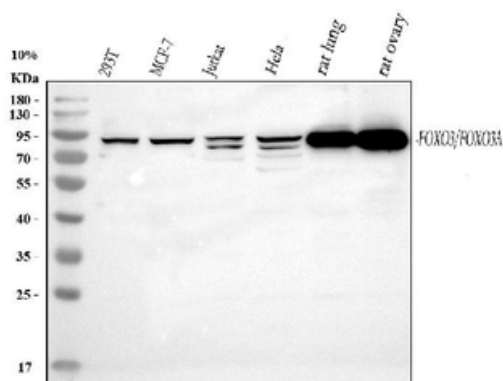
IHC analysis of FOXO3A using anti-FOXO3A antibody. FOXO3A was detected in a paraffin-embedded section of human pancreatic ductal adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 μ g/ml rabbit anti-FOXO3A Antibody overnight at 4°C. Peroxidase Conjugated Goat Anti-rabbit IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using HRP Conjugated Rabbit IgG Super Vision Assay Kit with DAB as the chromogen.

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Western blot analysis of FOXO3A using anti-FOXO3A antibody. Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: human 293T whole cell lysates, Lane 2: human MCF-7 whole cell lysates, Lane 3: human Jurkat whole cell lysates, Lane 4: human Hela whole cell lysates, Lane 5: rat lung tissue lysates, Lane 6: rat ovary tissue lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-FOXO3A antigen affinity purified polyclonal antibody at 0.5 μ g/mL overnight at 4°C, then washed with TBS-0.1% Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:5000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit with Tanon 5200 system. A specific band was detected for FOXO3A at approximately 80-90 kDa. The expected band size for FOXO3A is at 71 kDa.

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