Product Datasheet

Anti-Alpha Actinin 4/ACTN4 Antibody (orb371708)

Description	Anti-Alpha Actinin 4/ACTN4 Antibody
Species/Host	Rabbit
Reactivity	Human, Mouse, Rat
Conjugation	Unconjugated
Tested Applications	FC, ICC, IF, IHC, WB
Immunogen	E.coli-derived human Alpha Actinin 4 recombinant protein (Position: E561-V661). Human Alpha Actinin 4 shares 99% and 98% amino acid (aa) sequence identity with mouse and rat Alpha Actinin 4, respectively.
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, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5µg/ml, Human Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human Flow Cytometry (Fixed), 1-3 µg/1x106 cells, Human, Mouse, Rat. Add 0.2ml of distilled water will yield a concentration of 500ug/ml
Isotype	Rabbit IgG
Clonality	Polyclonal
Antibody Type	Primary Antibody
MW	105 kDa

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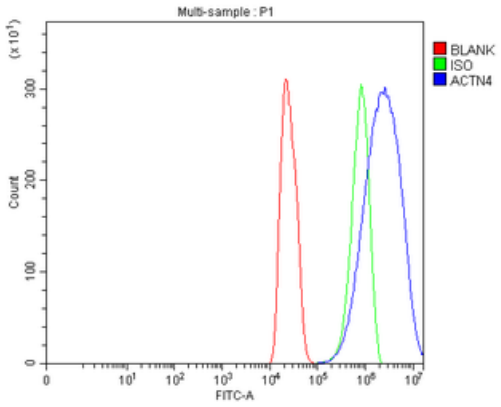
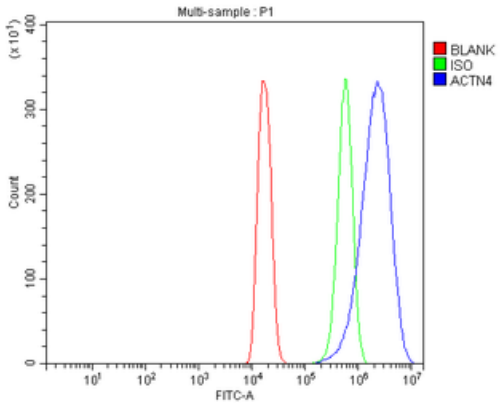
Uniprot ID O43707

Expiration Date 12 months from date of receipt.



Flow Cytometry analysis of C6 cells using anti-Alpha Actinin 4 antibody. Overlay histogram showing C6 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-Alpha Actinin 4 Antibody (1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



Flow Cytometry analysis of HEPA1-6 cells using anti-Alpha Actinin 4 antibody. Overlay histogram showing HEPA1-6 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-Alpha Actinin 4 Antibody (1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.

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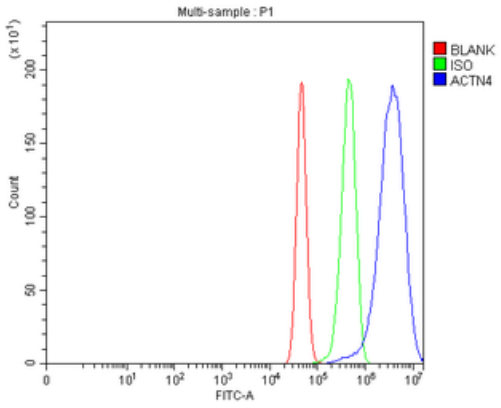
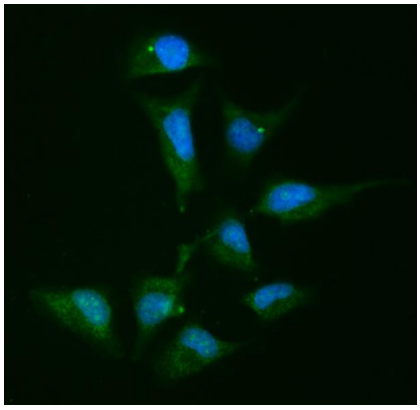
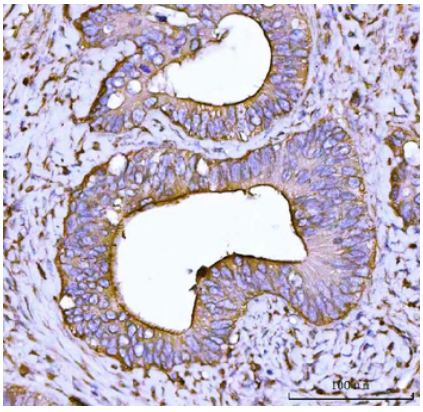


Flow Cytometry analysis of U87 cells using anti-Alpha Actinin 4 antibody. Overlay histogram showing U87 cells (Blue line). To facilitate intracellular staining, cells were fixed with 4% paraformaldehyde and permeabilized with permeabilization buffer. The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Alpha Actinin 4 Antibody (1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample without incubation with primary antibody and secondary antibody (Red line) was used as a blank control.



IF analysis of Alpha Actinin 4 using anti-Alpha Actinin 4 antibody. Alpha Actinin 4 was detected in an immunocytochemical section of Hela cells. 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 µg/mL rabbit anti-Alpha Actinin 4 Antibody overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.



IHC analysis of Alpha Actinin 4 using anti-Alpha Actinin 4 antibody. Alpha Actinin 4 was detected in a paraffin-embedded section of human colonic 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 µg/ml rabbit anti-Alpha Actinin 4 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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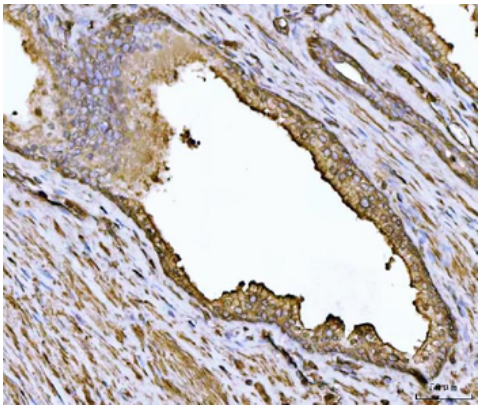
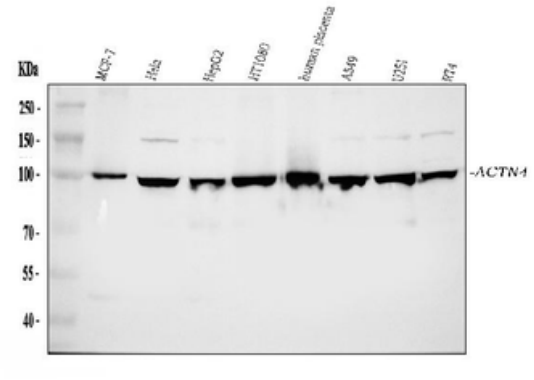


IHC analysis of Alpha Actinin 4 using anti-Alpha Actinin 4 antibody. Alpha Actinin 4 was detected in a paraffin-embedded section of human prostate cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml rabbit anti-Alpha Actinin 4 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.



Western blot analysis of Alpha Actinin 4 using anti-Alpha Actinin 4 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 MCF-7 whole cell lysates, Lane 2: human Hela whole cell lysates, Lane 3: human HepG2 whole cell lysates, Lane 4: huamn HT1080 whole cell lysates, Lane 5: human placenta tissue lysates, Lane 6: human A549 whole cell lysates, Lane 7: human U251 whole cell lysates, Lane 8: human RT-4 whole cell 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-Alpha Actinin 4 antigen affinity purified polyclonal antibody at 0.5 µg/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 Alpha Actinin 4 at approximately 105 kDa. The expected band size for Alpha Actinin 4 is at 105 kDa.

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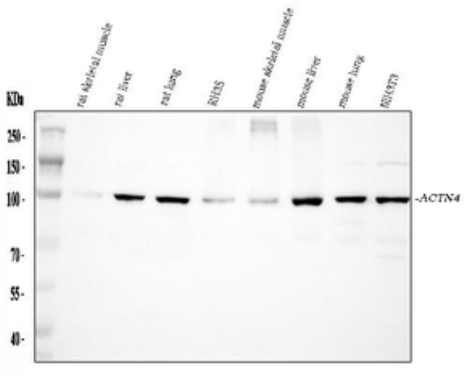

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