BOSTER
antibody and ELISA experts

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Anti-Alpha Actinin 4/ACTN4 Antibody Picoband™

Catalog Number: PB9974

About ACTN4

Alpha-actinin-4 is a protein that in humans is encoded by the ACTN4 gene. Alpha actinins belong to the spectrin gene superfamily which represents a diverse group of cytoskeletal proteins, including the alpha and beta spectrins and dystrophins. Alpha actinin is an actin-binding protein with multiple roles in different cell types. In nonmuscle cells, the cytoskeletal isoform is found along microfilament bundles and adherens-type junctions, where it is involved in binding actin to the membrane. In contrast, skeletal, cardiac, and smooth muscle isoforms are localized to the Z-disc and analogous dense bodies, where they help anchor the myofibrillar actin filaments. This ACTN4 gene encodes a nonmuscle, alpha actinin isoform which is concentrated in the cytoplasm, and thought to be involved in metastatic processes. Mutations in this gene have been associated with focal and segmental glomerulosclerosis.

Overview

Product Name	Anti-Alpha Actinin 4/ACTN4 Antibody Picoband™
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-Alpha Actinin 4/ACTN4 Antibody Picoband™ catalog # PB9974. Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human, Mouse, Rat.
Application	Flow Cytometry, IF, IHC, ICC, WB
Clonality	Polyclonal
Formulation	Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.
Storage Instructions	Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	O43707

Technical Details

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.
Predicted Reactive Species	Bovine
Recommended Detection Systems	Boster recommends Enhanced Chemiluminescent Kit with anti-Rabbit IgG (EK1002) for Western blot, and HRP Conjugated anti- Rabbit IgG Super Vision Assay Kit (SV0002-1) for IHC(P) and ICC.
Cross Reactivity	No cross-reactivity with other proteins
Isotype	Rabbit IgG

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Form	Lyophilized
Concentration	Adding 0.2 ml of distilled water will yield a concentration of 500 ug/ml.
Purification	Immunogen affinity purified.
Suggested Dilutions	Dilute the sample so that the expected range of concentrations fall within the detection range of this kit. If the expected range of concentration is unknown, a pilot test should be conducted to decide the optimal dilution ratio for your samples. Some PubMed article(s) citing the expression level of this target are as follows: Boster Bio's internal QC testing used: Western blot, 0.1-0.5ug/ml, Human, Mouse, Rat Immunohistochemistry (Paraffin-embedded Section), 2-5ug/ml, Human, By Heat Immunocytochemistry/Immunofluorescence, 5 ug/ml, Human Flow Cytometry, 1-3 ug/1x10^6 cells, Human, Mouse, Rat

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Anti-Alpha Actinin 4/ACTN4 Antibody Picoband™ (PB9974) Images

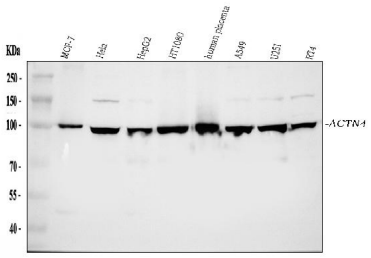

Figure 1. Western blot analysis of Alpha Actinin 4 using anti-Alpha Actinin 4 antibody (PB9974).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 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 (Catalog # PB9974) at 0.5 ug/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 (Catalog # EK1002) 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.

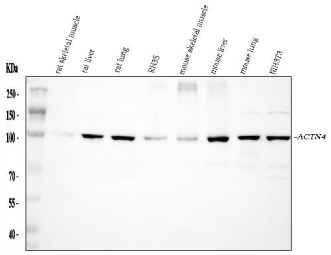

Figure 2. Western blot analysis of Alpha Actinin 4 using anti-Alpha Actinin 4 antibody (PB9974).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: rat skeletal muscle tissue lysates,
Lane 2: rai liver tissue lysates,
Lane 3: rat lung tissue lysates,
Lane 4: rat RH35 whole cell lysates,
Lane 5: mouse sketetal muscle tissue lysates,
Lane 6: mouse liver tissue lysates,
Lane 7: mouse lung tissue lysates,
Lane 8: mouse NIH/3T3 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 (Catalog # PB9974) at 0.5 ug/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 (Catalog # EK1002) with Tanon 5200

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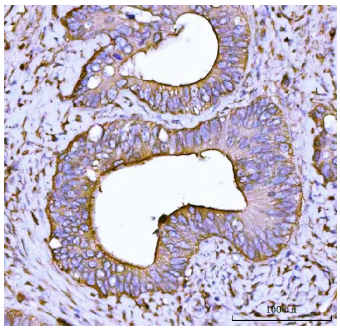

Figure 3. IHC analysis of Alpha Actinin 4 using anti-Alpha Actinin 4 antibody (PB9974).
Alpha Actinin 4 was detected in a paraffin-embedded section of human colonic adenocarcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-Alpha Actinin 4 Antibody (PB9974) 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 (Catalog # SV0002) with DAB as the chromogen.

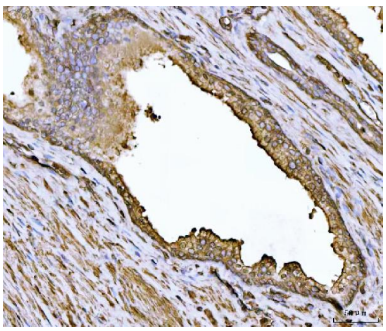

Figure 4. IHC analysis of Alpha Actinin 4 using anti-Alpha Actinin 4 antibody (PB9974).
Alpha Actinin 4 was detected in a paraffin-embedded section of human prostate cancer tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 ug/ml rabbit anti-Alpha Actinin 4 Antibody (PB9974) 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 (Catalog # SV0002) with DAB as the chromogen.

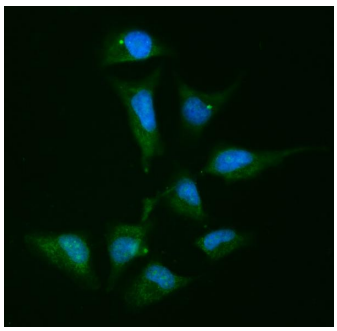

Figure 5. IF analysis of Alpha Actinin 4 using anti-Alpha Actinin 4 antibody (PB9974).
Alpha Actinin 4 was detected in an immunocytochemical section of Hela cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 ug/mL rabbit anti-Alpha Actinin 4 Antibody (PB9974) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Rabbit IgG (BA1127) 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.

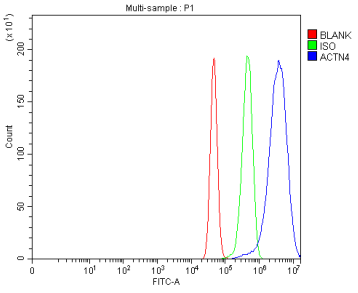

Figure 6. Flow Cytometry analysis of U87 cells using anti-Alpha Actinin 4 antibody (PB9974).
Overlay histogram showing U87 cells stained with PB9974 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Alpha Actinin 4 Antibody (PB9974, 1 ug/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10^6) used under the same conditions.

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Unlabelled sample (Red line) was also used as a control.

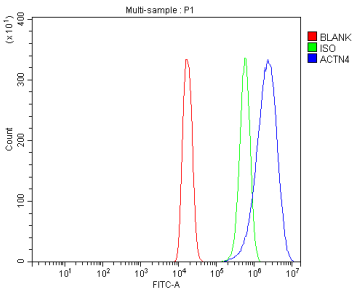

Figure 7. Flow Cytometry analysis of HEPA1-6 cells using anti-Alpha Actinin 4 antibody (PB9974).
Overlay histogram showing HEPA1-6 cells stained with PB9974 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Alpha Actinin 4 Antibody (PB9974, 1 ug/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

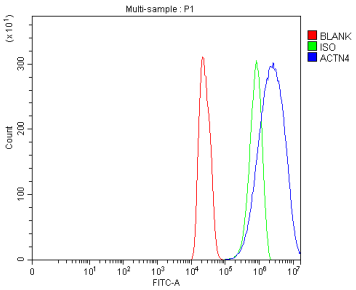

Figure 8. Flow Cytometry analysis of C6 cells using anti-Alpha Actinin 4 antibody (PB9974).
Overlay histogram showing C6 cells stained with PB9974 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with rabbit anti-Alpha Actinin 4 Antibody (PB9974, 1 ug/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-rabbit IgG (BA1127, 5-10 ug/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was rabbit IgG (1 ug/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.