Anti-beta Actin Antibody [PSH03-63]

HA722023

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, FC, IP
Molecular Wt:	Predicted band size: 42 kDa
Clone number:	PSH03-63

Description: Actins are highly conserved proteins involved in cell motility, structure and integrity. Actin has been found to be expressed in at least six isomeric forms. It is expressed in heart and skeletal striated muscle tissue, and in certain smooth muscle tissues, regulating contractile potentials for these cells. It is also expressed in the cytoplasm of non-muscle cells, functioning to control cell structure and motility. Beta actin is usually used as a loading control, for among others, the integrity of cells, protein degradation, in Western Blotting.

Immunogen: Synthetic peptide within N-terminal residues of β-Actin.

Positive control: HeLa cell lysate, HepG2 cell lysate, MCF7 cell lysate, A431 cell lysate, Jurkat cell lysate, HEK-293 cell lysate, RAW264.7 cell lysate, C2C12 cell lysate, PC-12 cell lysate, mouse testis tissue lysate, mouse spleen tissue lysate, rat testis tissue lysate, rat kidney tissue lysate, HeLa, NIH/3T3, C6.

Subcellular location: Cytoskeleton.

Database links: SwissProt: P60709 Human | P60710 Mouse | P60711 Rat

Recommended Dilutions:

WB	1:20,000-1:640,000
IF-Cell	1:100-1:250
FC	1:1,000
IP	1-2μg/sample

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

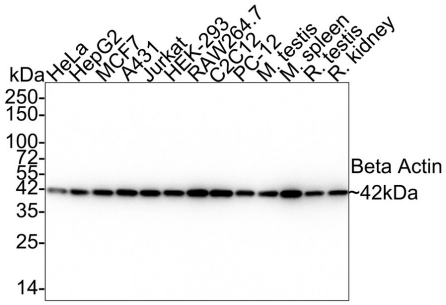

Fig1: Western blot analysis of beta Actin on different lysates with Rabbit anti-beta Actin antibody (HA722023) at 1/20,000 dilution.

Lane 1: HeLa cell lysate (10 µg/Lane)
Lane 2: HepG2 cell lysate (10 µg/Lane)
Lane 3: MCF7 cell lysate (10 µg/Lane)
Lane 4: A431 cell lysate (10 µg/Lane)
Lane 5: Jurkat cell lysate (10 µg/Lane)
Lane 6: HEK-293 cell lysate (10 µg/Lane)
Lane 7: RAW264.7 cell lysate (10 µg/Lane)
Lane 8: C2C12 cell lysate (10 µg/Lane)
Lane 9: PC-12 cell lysate (10 µg/Lane)
Lane 10: Mouse testis tissue lysate (10 µg/Lane)
Lane 11: Mouse spleen tissue lysate (10 µg/Lane)
Lane 12: Rat testis tissue lysate (10 µg/Lane)
Lane 13: Rat kidney tissue lysate (10 µg/Lane)

Predicted band size: 42 kDa
Observed band size: 42 kDa

Exposure time: 3 seconds;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722023) at 1/20,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

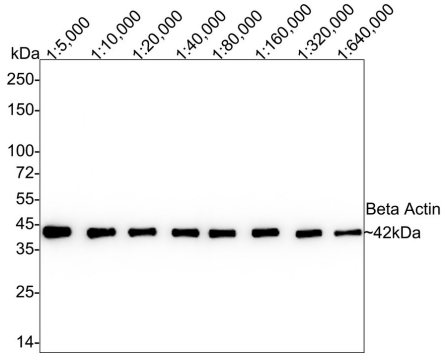

Fig2: Western blot analysis of beta Actin on HepG2 cell lysates with Rabbit anti-beta Actin antibody (HA722023) at different dilutions.

Lysates/proteins at 20 µg/Lane.

Predicted band size: 42 kDa
Observed band size: 42 kDa

Exposure time: 30 seconds;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA722023) at different dilutions was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

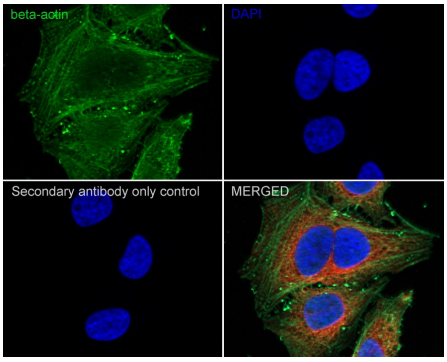

Fig3: Immunocytochemistry analysis of HeLa cells labeling beta Actin with Rabbit anti-beta Actin antibody (HA722023) at 1/250 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-beta Actin antibody (HA722023) at 1/250 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

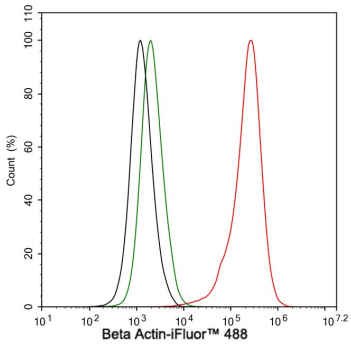

Fig4: Flow cytometric analysis of HeLa cells labeling beta Actin.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722023, 1µg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

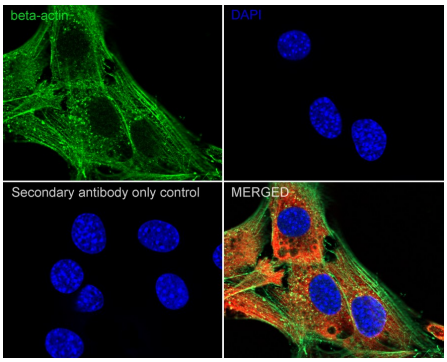

Fig5: Immunocytochemistry analysis of NIH/3T3 cells labeling beta Actin with Rabbit anti-beta Actin antibody (HA722023) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-beta Actin antibody (HA722023) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

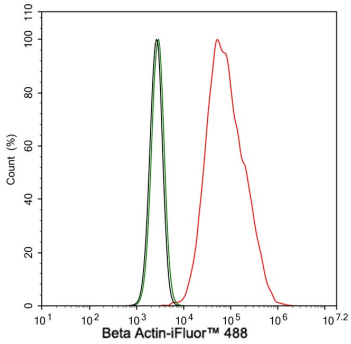

Fig6: Flow cytometric analysis of NIH/3T3 cells labeling beta Actin.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722023, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

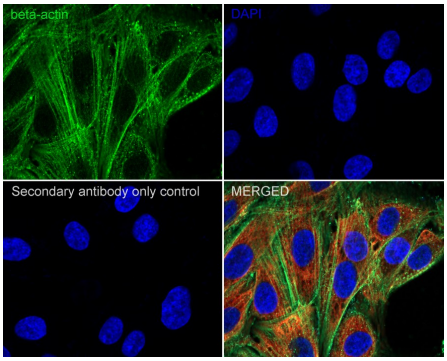

Fig7: Immunocytochemistry analysis of L6 cells labeling beta Actin with Rabbit anti-beta Actin antibody (HA722023) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-beta Actin antibody (HA722023) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

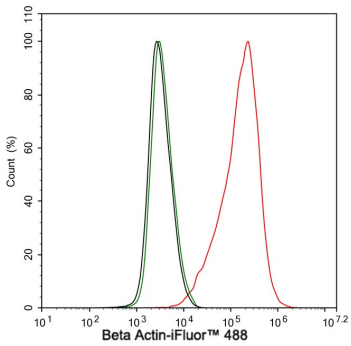

Fig8: Flow cytometric analysis of C6 cells labeling beta Actin.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722023, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

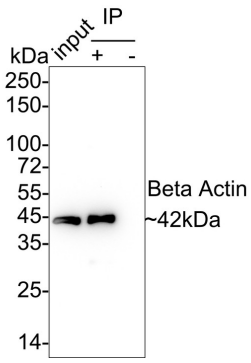

Fig9: beta Actin was immunoprecipitated from 0.2 mg NIH/3T3 cell lysate with HA722023 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using HA722023 at 1/5,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: NIH/3T3 cell lysate (input)
Lane 2: HA722023 IP in NIH/3T3 cell lysate
Lane 3: Rabbit IgG instead of HA722023 in NIH/3T3 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 1 minute 2 seconds; ECL: K1801

Background References

1. Ponte P., Ng S.Y., Engel J., Gunning P., Kedes L."Evolutionary conservation in the untranslated regions of actin mRNAs: DNA sequence of a human beta-actin cDNA." Nucleic Acids Res. 12:1687-1696(1984) Ohmori H., Toyama S., Toyama S."Direct proof that the primary site of action of cytochalasin on cell motility processes is actin."J. Cell Biol. 116:933-941(1992)