Anti-Phospho-Beta Catenin (S552) Antibody [PSH08-72] - BSA and Azide free

HA751237

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IHC-P, FC
Molecular Wt:	Predicted band size: 85 kDa
Clone number:	PSH08-72

Description: The cellular level of β-catenin is mostly controlled by its ubiquitination and proteosomal degradation. The E3 ubiquitin ligase TrCP1 (also known as β-TrCP) can recognize β-catenin as its substrate through a short linear motif on the disordered N-terminus. However, this motif (Asp-Ser-Gly-Ile-His-Ser) of β-catenin needs to be phosphorylated on the two serines in order to be capable to bind β-TrCP. Phosphorylation of the motif is performed by Glycogen Synthase Kinase 3 alpha and beta (GSK3α and GSK3β). GSK3s are constitutively active enzymes implicated in several important regulatory processes. There is one requirement, though: substrates of GSK3 need to be pre-phosphorylated four amino acids downstream (C-terminally) of the actual target site. Thus it also requires a "priming kinase" for its activities. In the case of β-catenin, the most important priming kinase is Casein Kinase I (CKI). Once a serine-threonine rich substrate has been "primed", GSK3 can "walk" across it from C-terminal to N-terminal direction, phosphorylating every 4th serine or threonine residues in a row. This process will result in dual phosphorylation of the aforementioned β-TrCP recognition motif as well.

Immunogen: Synthetic phosphopeptide corresponding to residues surrounding Ser552 of Human Beta catenin.

Positive control: HeLa cell lysate, HeLa treated with 10μM MG-132 for 6 hours cell lysate, NIH/3T3 cell lysate, NIH/3T3 treated with 10μM MG-132 for 8 hours cell lysate, C6 cell lysate, mouse colon tissue, C6 cells treated with 25μM MG-132 for 4 hours, human colon tissue, mouse liver tissue, rat colon tissue, NIH/3T3, C6.

Subcellular location: Cytoplasm, Nucleus, Cell membrane.

Database links: SwissProt: P35222 Human | Q02248 Mouse | Q9WU82 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100
IHC-P	1:200
FC	1:1,000

Storage Buffer: PBS (pH7.4).

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

Purity: Protein A affinity purified.

Images

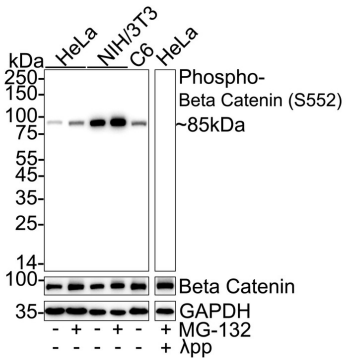

Fig1: Western blot analysis of Phospho-Beta Catenin (S552) on different lysates with Rabbit anti-Phospho-Beta Catenin (S552) antibody (HA751237) at 1/2,000 dilution and pan Beta Catenin antibody (ET1601-5) at 1/2,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 10μM MG-132 for 6 hours cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: NIH/3T3 treated with 10μM MG-132 for 8 hours cell lysate
Lane 5: C6 cell lysate
Lane 6: HeLa treated with 10μM MG-132 for 6 hours cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 20 μg/Lane.

Predicted band size: 85 kDa
Observed band size: 85 kDa

Exposure time: 30 seconds; ECL: K1802;

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 (HA751237) at 1/2,000 dilution and pan Beta Catenin antibody (ET1601-5) at 1/2,000 dilution were 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.

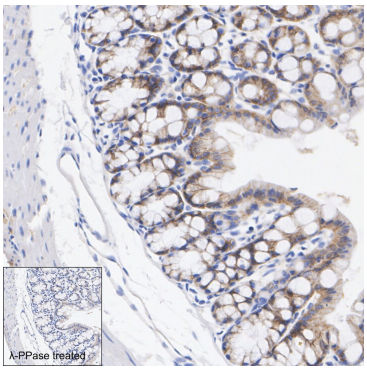

Fig2: Immunohistochemical analysis of paraffin-embedded mouse colon tissue with Rabbit anti-Phospho-Beta Catenin (S552) antibody (HA751237) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA751237) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

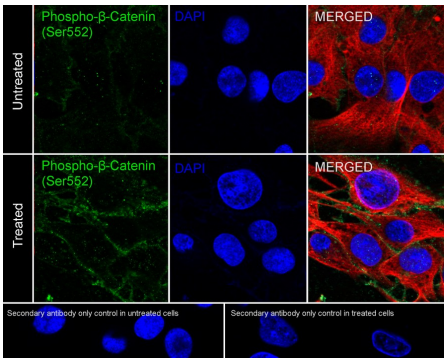

Fig3: Immunocytochemistry analysis of C6 cells treated with 25μM MG-132 for 4 hours labeling Phospho-Beta Catenin (S552) with Rabbit anti-Phospho-Beta Catenin (S552) antibody (HA751237) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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-Phospho-Beta Catenin (S552) antibody (HA751237) 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 (HA601187, 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.

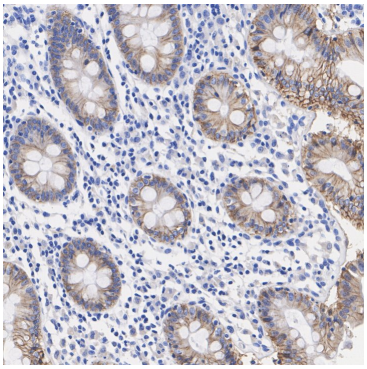

Fig4: Immunohistochemical analysis of paraffin-embedded human colon tissue with Rabbit anti-Phospho-Beta Catenin (S552) antibody (HA751237) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA751237) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

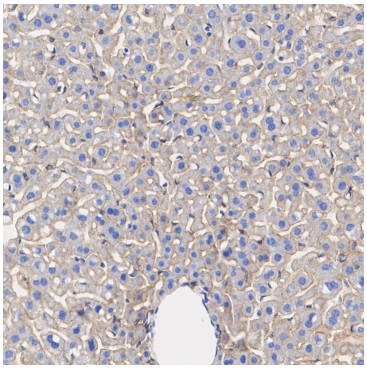

Fig5: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-Phospho-Beta Catenin (S552) antibody (HA751237) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA751237) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

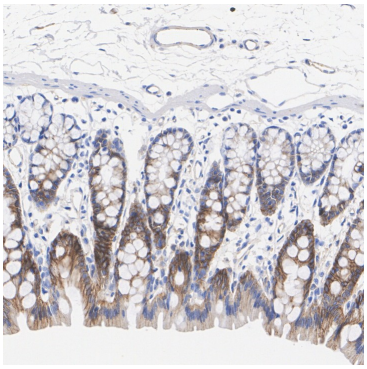

Fig6: Immunohistochemical analysis of paraffin-embedded rat colon tissue with Rabbit anti-Phospho-Beta Catenin (S552) antibody (HA751237) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA751237) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

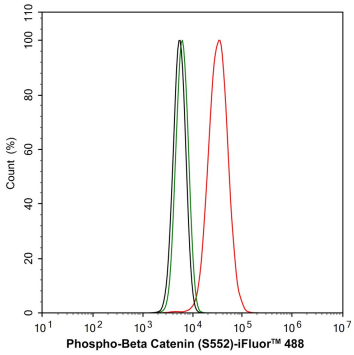

Fig7: Flow cytometric analysis of NIH/3T3 cells labeling Phospho-Beta Catenin (S552).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA751237, 1/1,000) (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).

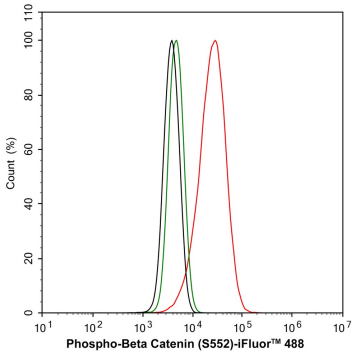

Fig8: Flow cytometric analysis of C6 cells labeling Phospho-Beta Catenin (S552).

Cells were fixed and permeabilized. Then stained with the primary antibody (HA751237, 1/1,000) (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).

Background References

1. Liu J et al. Wnt/beta-catenin signalling: function, biological mechanisms, and therapeutic opportunities. Signal Transduct Target Ther. 2022 Jan
2. Yu F et al. Wnt/beta-catenin signaling in cancers and targeted therapies. Signal Transduct Target Ther. 2021 Aug