Anti-Phospho-Beta Catenin (S675) Antibody [PSH25-60] - BSA and Azide free

HA751979

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-Fr, IHC-P, IP
Molecular Wt:	Predicted band size: 85 kDa
Clone number:	PSH25-60

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 Ser675 of Human Beta catenin.
Positive control:	Mouse brain tissue lysate, Mouse liver tissue lysate, Rat brain tissue lysate, Rat liver tissue lysate.
Subcellular location:	Cytoplasm, Nucleus, Cell membrane.
Database links:	SwissProt: P35222 Human | Q02248 Mouse | Q9WU82 Rat
Recommended Dilutions:	
WB	1:5,000
IHC-Fr	1:1,000
IHC-P	1:4,000
IP	1-2μg/sample
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

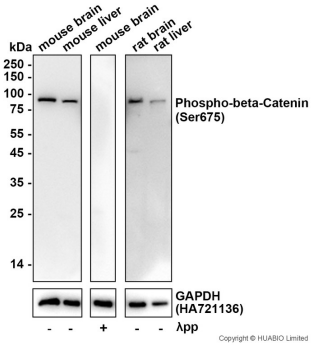

Fig1: Western blot analysis of Phospho-Beta Catenin (S675) on different lysates with Rabbit anti-Phospho-Beta Catenin (S675) antibody (HA751979) at 1/5,000 dilution.

Lane 1: Mouse brain tissue lysate
Lane 2: Mouse liver tissue lysate
Lane 3: Mouse brain tissue lysate, the membrane treated with λpp for 1 hour
Lane 4: Rat brain tissue lysate
Lane 5: Rat liver tissue lysate

Lysates/proteins at 20 µg/Lane.
Exposure time: Lane 1-3: 1 minute; Lane 4-5: 2 minutes; ECL: K1801

Blocking: 5% NFDM/TBST, 1 hour at room temperature
Primary antibody: HA751979, 1/5,000 in primary antibody dilution buffer (K1803), overnight at 4 °C
Secondary antibody: Goat anti-Rabbit IgG-HRP (HA1001), 1/50,000 in 5% NFDM/TBST, 1 hour at room temperature

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

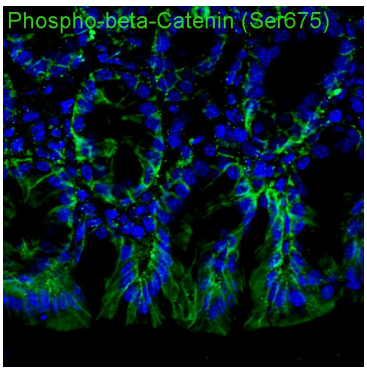

Fig2: Application: Immunofluorescence (IHC-Fr)

Species: Mouse
Tissue: Colon
Sample: Frozen section

Antigen retrieval: Not required

Wash buffer: 1× TBST
Blocking: 10% normal goat serum + 1% Triton X-100 + 0.3 M Glycine in TBST, 30 minutes at room temperature.

Primary antibody: HA751979, 1/1,000, overnight at 4°C.
Secondary antibody: Goat Anti-Rabbit IgG (iFluor™ 488, HA1121), 1.5 hours at room temperature.

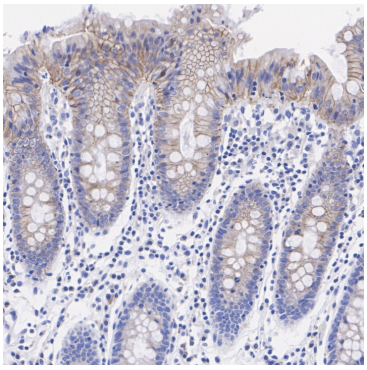

Fig3: Application: Immunohistochemistry (IHC-P)

Species: Human
Tissue: Colon
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST
Endogenous peroxidase blocking: 3% H_2O_2, 10 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: HA751979, 1/4,000, 1 hour at room temperature.
Secondary antibody: HA1119, 20 minutes at room temperature.

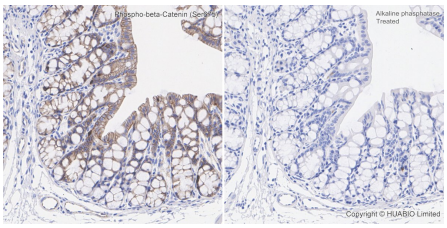

Fig4: Application: Immunohistochemistry (IHC-P)

Species: Mouse
Tissue: Colon
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST
Endogenous peroxidase blocking: 3% H_2O_2, 10 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: HA751979, 1/4,000, 1 hour at room temperature.
Secondary antibody: HA1119, 20 minutes at room temperature.

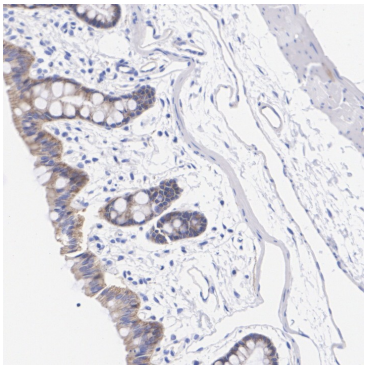

Fig5: Application: Immunohistochemistry (IHC-P)

Species: Rat
Tissue: Colon
Sample: Paraffin-embedded section

Antigen retrieval: Heat-mediated, Tris-EDTA buffer (pH 9.0), 20 minutes at 95°C.

Wash buffer: 1× TBST
Endogenous peroxidase blocking: 3% H_2O_2, 10 minutes at room temperature.
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.
Primary antibody: HA751979, 1/4,000, 1 hour at room temperature.
Secondary antibody: HA1119, 20 minutes at room temperature.

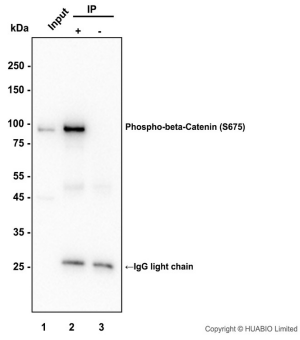

Fig6: Immunoprecipitation (IP)

Phospho-Beta Catenin (S675) was immunoprecipitated in 0.2 mg mouse brain tissue lysate with HA751979 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA751979 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: Mouse brain tissue lysate (input)
Lane 2: HA751979 IP in mouse brain tissue lysate
Lane 3: Rabbit IgG instead of HA751979 in mouse brain tissue lysate

Exposure time: 25 seconds
Blocking: 5% NFDM/TBST, 1 hour at room temperature

Primary dilution: HA751979, 1/5,000 in primary antibody dilution buffer (K1803), 2 hours at room temperature

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

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