

# Anti-Phospho-Beta Catenin (S675) Antibody [PSH25-60]

## HA724534



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

**Description:** The cellular level of  $\beta$ -catenin is mostly controlled by its ubiquitination and proteosomal degradation. The E3 ubiquitin ligase TrCP1 (also known as  $\beta$ -TrCP) can recognize  $\beta$ -catenin as its substrate through a short linear motif on the disordered N-terminus. However, this motif (Asp-Ser-Gly-Ile-His-Ser) of  $\beta$ -catenin needs to be phosphorylated on the two serines in order to be capable to bind  $\beta$ -TrCP. Phosphorylation of the motif is performed by Glycogen Synthase Kinase 3 alpha and beta (GSK3 $\alpha$  and GSK3 $\beta$ ). 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  $\beta$ -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  $\beta$ -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:**

<b>WB</b>	1:5,000
<b>IHC-Fr</b>	1:1,000
<b>IHC-P</b>	1:4,000
<b>IP</b>	1-2 $\mu$ g/sample

**Storage Buffer:** 1\*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.

**Storage Instruction:** Shipped at 4 $^{\circ}$ C. Store at +4 $^{\circ}$ C short term (1-2 weeks). Store at -20 $^{\circ}$ C long term.

**Purity:** Protein A affinity purified.

Hangzhou Huaan Biotechnology Co., Ltd.

Orders:0086-571-88062880

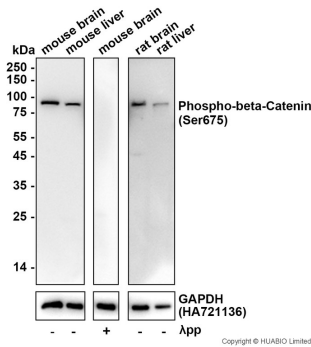
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## Images

**Fig1:** Western blot analysis of Phospho-Beta Catenin (S675) on different lysates with Rabbit anti-Phospho-Beta Catenin (S675) antibody (HA724534) 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  $\lambda$ pp for 1 hour

Lane 4: Rat brain tissue lysate

Lane 5: Rat liver tissue lysate

Lysates/proteins at 20  $\mu$ g/Lane.

Exposure time: Lane 1-3: 1 minute; Lane 4-5: 2 minutes; ECL: K1801

Blocking: 5% NFDm/TBST, 1 hour at room temperature

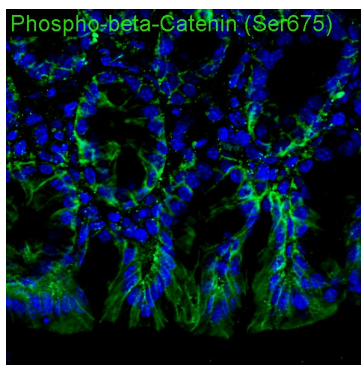
Primary antibody: HA724534, 1/5,000 in primary antibody dilution buffer (K1803), overnight at 4  $^{\circ}$ C

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 $\times$  TBST

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

Primary antibody: HA724534, 1/1,000, overnight at 4 $^{\circ}$ C.

Secondary antibody: Goat Anti-Rabbit IgG (iFluor<sup>TM</sup> 488, HA1121), 1.5 hours at room temperature.

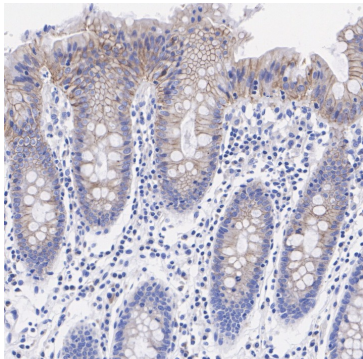
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**Fig3:** Application: Immunohistochemistry (IHC-P)

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<sub>2</sub>O<sub>2</sub>, 10 minutes at room temperature.

Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.

Primary antibody: HA724534, 1/4,000, 1 hour at room temperature.

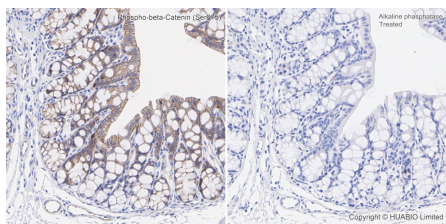
Secondary antibody: HA1119, 20 minutes at room temperature.

**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.

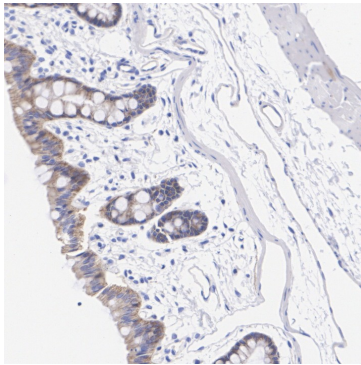
Wash buffer: 1× TBST

Endogenous peroxidase blocking: 3% H<sub>2</sub>O<sub>2</sub>, 10 minutes at room temperature.

Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.

Primary antibody: HA724534, 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<sub>2</sub>O<sub>2</sub>, 10 minutes at room temperature.

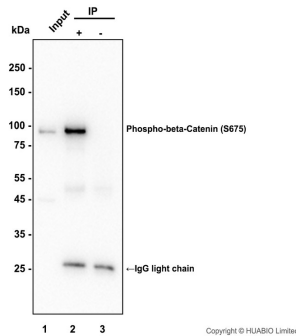
Blocking: 1% BSA + 10% normal goat serum, 10 minutes at room temperature.

Primary antibody: HA724534, 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 HA724534 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using HA724534 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: HA724534 IP in mouse brain tissue lysate

Lane 3: Rabbit IgG instead of HA724534 in mouse brain tissue lysate

Exposure time: 25 seconds

Blocking: 5% NFDM/TBST, 1 hour at room temperature

Primary dilution: HA724534, 1/5,000 in primary antibody dilution buffer (K1803), 2 hours at room temperature

Predicted band size: 85 kDa

Observed band size: 85 kDa

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**Note:** All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

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### 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

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