Anti-Phospho-Beta Catenin (T41/S45) Antibody [JE54-02]

HA722316

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P
Molecular Wt:	Predicted band size: 85 kDa
Clone number:	JE54-02

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 Thr41/Ser45 of human Beta catenin.

Positive control: HEK-293 treated with 200nM Calyculin A for 1 hour cell lysate, NIH/3T3 treated with 100nM Calyculin A for 30 minutes cell lysate, C6 treated with 100nM Calyculin A for 30 minutes cell lysate, human kidney tissue, mouse kidney tissue, rat kidney tissue.

Subcellular location: Cytoplasm, Nucleus, Cell membrane.

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

Recommended Dilutions:

WB	1:1,000
IHC-P	1:200

Storage Buffer: 1*TBS (pH7.4), 0.05% 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

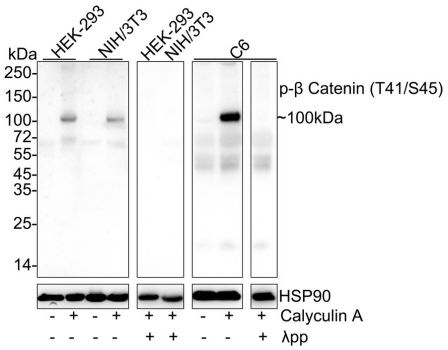

Fig1: Western blot analysis of Phospho-Beta Catenin (T41/S45) on different lysates with Rabbit anti-Phospho-Beta Catenin (T41/S45) antibody (HA722316) at 1/1,000 dilution.

Lane 1: HEK-293 cell lysate
Lane 2: HEK-293 treated with 200nM Calyculin A for 1 hour cell lysate
Lane 3: NIH/3T3 cell lysate
Lane 4: NIH/3T3 treated with 100nM Calyculin A for 30 minutes cell lysate
Lane 5: HEK-293 treated with 200nM Calyculin A for 1 hour cell lysate, then the membrane treated with λpp for 1 hour
Lane 6: NIH/3T3 treated with 100nM Calyculin A for 30 minutes cell lysate, then the membrane treated with λpp for 1 hour
Lane 7: C6 cell lysate
Lane 8: C6 treated with 100nM Calyculin A for 30 minutes cell lysate
Lane 9: C6 treated with 100nM Calyculin A for 30 minutes cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 30 µg/Lane.

Predicted band size: 85 kDa
Observed band size: 100 kDa
Exposure time: Lane 1-4: 3 minutes; Lane 5-9: 1 minute; 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 (HA722316) at 1/1,000 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.

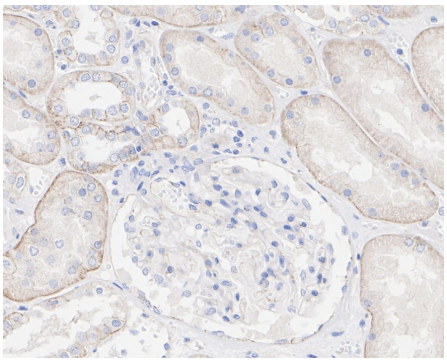

Fig2: Immunohistochemical analysis of paraffin-embedded human kidney tissue with Rabbit anti-Phospho-Beta Catenin (T41/S45) antibody (HA722316) at 1/200 dilution.

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 (HA722316) 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.

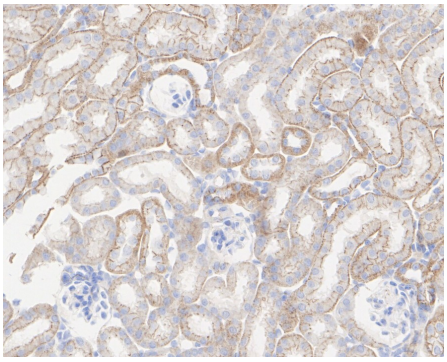

Fig3: Immunohistochemical analysis of paraffin-embedded mouse kidney tissue with Rabbit anti-Phospho-Beta Catenin (T41/S45) antibody (HA722316) at 1/200 dilution.

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 (HA722316) 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.

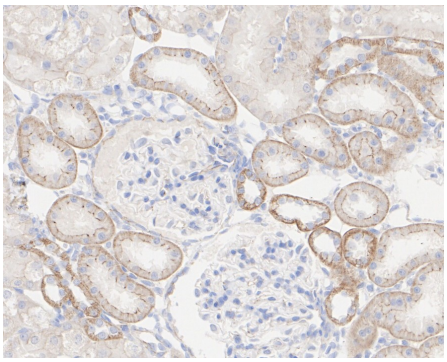

Fig4: Immunohistochemical analysis of paraffin-embedded rat kidney tissue with Rabbit anti-Phospho-Beta Catenin (T41/S45) antibody (HA722316) at 1/200 dilution.

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 (HA722316) 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.

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

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