SYMANSIS
CELL SIGNALING SCIENCE

β-Catenin, (active, dephosphorylated) antibody

Catalog Number 3024DP

Applications
Western blot, Immunohistochemistry

Species cross reactivity
Human, Mouse, Rat other species not tested.

Molecular weight
92 kDa, SDS-reducing gels.

Background
This antibody was designed to detect the active form of β-catenin. The antibody exhibits a strong preference for dephosphorylated beta catenin protein by western blot, but the antibody may show cross reactivity with phosphorylated β-catenin under certain conditions.

β-catenin is part of the cadherin cell adhesion complex and central to the Wingless/Wnt signalling pathway. Wnt signalling results in β-catenin accumulation and transcriptional activation of specific target genes. In the absence of Wnt signaling, β-Catenin is phosphorylated by GSK-3, leading to its ubiquitination and subsequent degradation by the proteasome. Thus, at steady state in the absence of Wnt signaling, β-catenin is rapidly degraded in the cytoplasm. Nuclear levels of β-catenin are kept low by APC and axin interactions, both of which have a nuclear export activity that shuttles β-catenin into the cytoplasm.

Immunogen: CQSYLDSGIHSGATTTAPSL, corresponding to the region surrounding the phosphorylated amino acids of beta catenin at S33, 37, and T41 (highlighted in red). The peptide maps to amino acids 28 to 46 of human beta catenin.

NCBI protein accession number
P35222

Source and purification
Polyclonal antibodies are produced by immunizing sheep with a synthetic peptide (KLH-coupled), followed by affinity absorption over β-Catenin phosphorylated peptides and affinity purification over the non-phosphorylated peptide specific for β-Catenin.

Storage and use
100 µg supplied as lyophilised protein in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, and 0.05 % sodium azide. Store at 4 °C. Reconstitute in 100 µl of sterile water. This product is for in vitro use only. Add 1 mg/mL high quality BSA or glycerol before freezing. Avoid multiple freeze thaw cycles.

Recommended antibody dilutions
Western blotting	2 µg/mL
Immunocytochemistry	4 µg/mL

FOR RESEARCH USE ONLY

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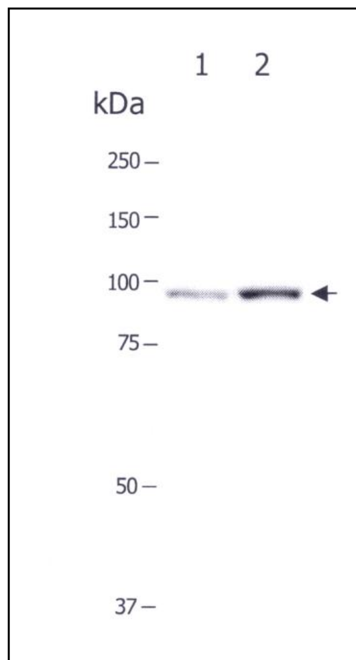
Protocols *(suggested protocols, optimal conditions must be determined by enduser)*

Western Blot

For Western blot analysis, block membrane with 5 % w/v milk, 1x TBS and 0.1% Tween-20 (TBST) for 30 min at room temperature and incubate with diluted antibody (in 1 % w/v BSA, TBST) at 4 °C overnight with gentle rocking. Wash 3X with at least 50 mL for 15 minutes each with 1X TBST. Incubate secondary detection antibody 1:10,000 to 1:100,000 depending upon brand for 30 min at room temperature. Wash 4X with 50 mL of TBST for 15 min at room temperature. Develop with luminescent substrate as required.



Western blot analysis of extracts from HepG2 cells using 3024DP at 2 µg/mL. Arrow indicates β-catenin. Untreated (lane 1) or treated with GSK3 inhibitor (lane 2). As expected, there is an increase in dephosphorylated β-catenin in the presence of the GSK inhibitor demonstrating the enhance preference of 3024DP for non-phosphorylated β-catenin

Immunohistochemistry:
Staining on INS-1E Cells, an example.

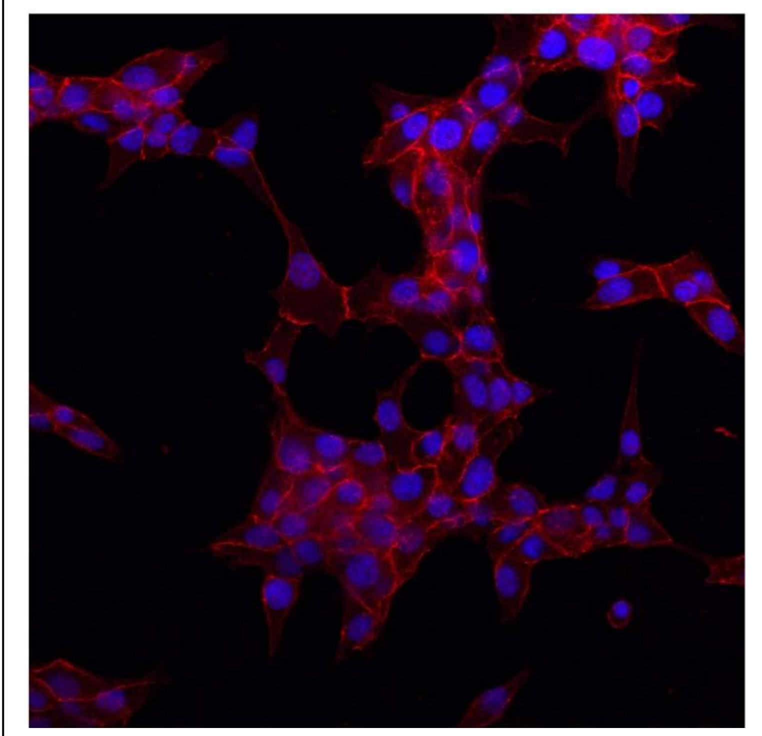
A. INS-E cell culture

1. INS-E cells were trypsinized and seeded into BD 8-chamber glass slides ($85x10^3$ cells per chamber instandard 300 µl RPMI), already coated with collagen-1 (collagen-1 conc = 10µg/ml in 0.02 M acetic acid; sterile filtered before use). Coating done in the tissue culture lab, stored in the fridge until ready to use; note: chamber cells are submerged in excess PBS during storage.
2. Cells cultured for approximately 48hr (with 1 media change after 24 hr)
3. Media aspirated and cells fixed with freshly prepared 4% PFA for 15 min, washed 3x with PBS and stored in PBS at 40C until ready to perform immunostaining.

B. INS-1E immunostaining

1. PBS from chamber slides aspirated and cells permeabilized with 0.2% Triton-X100/PBS for 5-6 min.
2. Triton-X100 aspirated and washed 3x with PBS
3. Cells blocked with 5% normal donkey serum (NDS)/PBS, 1 hr RT
4. NDS aspirated and cells incubated with ovine anti-β-catenin (active, catalogue number 3024DP), diluted to 4µg/mL with 1% NDS/PBS, overnight at 4°C.
5. Following washing with excess PBS (3X), cells incubated with donkey anti-sheep IgG-Alexa 568 (diluted 600x with 1% NDS/PBS), 1 hr RT

6. Secondary antibody aspirated and cells washed with excess PBS (4x)
7. Upper plastic chamber removed, slide mounted with ProlongGold/DAPI.
8. Examined and imaged by confocalmicroscopy (405 nm (DAPI) and 568 nm (Alexa 568) excitation, Z-series)



Staining of 3024DP, localization is right at the junctions between cells as expected

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