

# Anti-β-Catenin

Polyclonal Antibody

Cat. # 06-734

Lot # DAM1614918

pack size: 200 µg

Store at -20°C

FOR RESEARCH USE ONLY  
NOT FOR USE IN HUMANS



## Certificate of Analysis

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Applications	Species Cross-Reactivity	Antibody Isotype	Epitope/Region	Host Species	Molecular Weight	Accession #
WB, IP, IC	B, H, M, R, Rb	IgG	N/A	Rb	92 kDa	NP_001091679

### Background

β-catenin plays a central role in cell adhesion, bridging between cadherins and actin cytoskeleton. These activities are regulated through its differential interaction with several molecular partners, including cell-cell adherens junction (AJ) molecules, components of its degradation complex, and transcription factors.

β-Catenin is composed of three domains: a central armadillo repeat domain and two N- and C-terminal tails. β-catenin can be phosphorylated by serine-threonine kinase, such as GSK-3. The phosphorylated β-catenin affects the protein association of β-catenin to E-cadherin and α-catenin that occurs in the cell. This process can be reversed by dephosphorylation of β-catenin.

### Presentation

Purified rabbit polyclonal IgG in buffer containing 0.1 M Tris-glycine, pH 7.4, 0.15 M NaCl, 0.05% sodium azide and 30% glycerol. Store at -20°C.

### Concentration

1 mg/mL

### Specificity

Recognizes β-Catenin, Mr 92 kDa.

### Species Cross-reactivity

Human, bovine, rat, rabbit and mouse.

### Immunogen

KLH-conjugated, synthetic peptide (CGG-SYLDSGIHSGATTAPSLSGK) corresponding to the consensus GSK3 phosphorylation site of human β-Catenin (amino acids 29-49).

### Molecular Weight

92 kDa

### Method of Purification

Protein A chromatography

### Storage and Handling

Stable for 1 year at -20°C from date of receipt.

Handling Recommendations: Upon first thaw, and prior to removing the cap, centrifuge the vial and gently mix the solution. Aliquot into microcentrifuge tubes and store at -20°C. Avoid repeated freeze/thaw cycles, which may damage IgG and affect product performance. Note: Variability in freezer temperatures below -20°C may cause glycerol containing solutions to become frozen during storage.

### Control

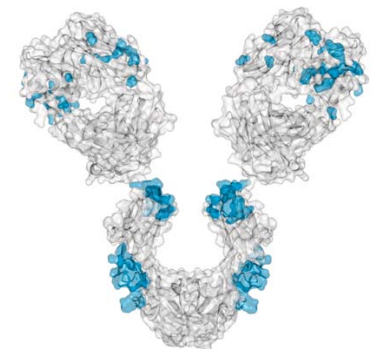
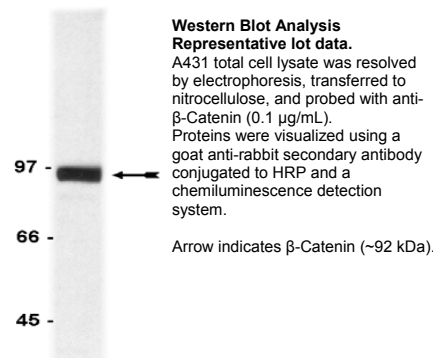
A431 cell lysate

**Included Positive Antigen Control:** Catalog #12-301, Non stimulated A431 cell lysate. Add 2.5 µL of 2-mercaptoethanol/100 µL of lysate and boil for 5 minutes to reduce the preparation. Load 20 µg of reduced lysate per lane for minigels.

### Quality Control Testing

Routinely evaluated by western blot on A431 total cell lysate.

**Western Blot Analysis:** 0.1-1 µg/mL of this lot detected β-catenin in an A431 total cell lysate.



### References

1. Shimizu, H., et al. (1997). *Cell Growth Differ.* 8: 1349-1358.
2. Knudsen, K. A., et al. (1995). *J. Cell. Biol.* 130: 67-77.

### Additional Research Applications

**Immunoprecipitation:** 5 µg of a previous lot immunoprecipitated β-Catenin from 500 µg of A431 membrane fraction lysate.

**Immunocytochemistry:** 1-5 µg/mL of a previous lot showed positive immunostaining for β-catenin at cell-cell junctions of A431 cells fixed with 4% paraformaldehyde and permeabilized with 0.25% Triton® X-100.

**APPLICATION LEGEND:** WB Western Blotting IP Immunoprecipitation IC Immunocytochemistry IF Immunofluorescence  
IH Immunohistochemistry (Tissue)

**SPECIES LEGEND:** B Bovine H Human M Mouse R Rat Rb Rabbit WR Most Common Vertebrates

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**PROTOCOL****Western Blot**

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EDTA; 1 mM PMSF; 1  $\mu$ g/mL each aprotinin, leupeptin, pepstatin; 1 mM Na<sub>3</sub>VO<sub>4</sub>, 1 mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), (PBS-MLK) for 20-30 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with **0.1-1  $\mu$ g/mL of anti- $\beta$ -Catenin**, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
6. Wash the nitrocellulose with water twice.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Use detection method of choice (enhanced chemiluminescence was used).

**Immunoprecipitation**

1. Add **5  $\mu$ g of anti- $\beta$ -Catenin** and 60  $\mu$ L (30  $\mu$ L packed beads) of washed Protein A agarose bead slurry (Catalog # 16-125) to 500  $\mu$ L of PBS in a microcentrifuge tube.
2. Gently rock the reaction mixture at 4°C for 2 hours.
3. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
4. Dilute the cell lysate to roughly 1  $\mu$ g/ $\mu$ L total cell protein with PBS.
5. Add 500  $\mu$ g-1mg cell lysate to the reaction mixture.
6. Gently rock the reaction mixture at 4°C for 1 hour.
7. Collect the agarose beads by pulsing (5 seconds in the microcentrifuge at 14,000 x g), and drain off the supernatant. Wash the beads 3 times with either ice-cold cell lysis buffer or PBS.
8. Resuspend the agarose beads in 60  $\mu$ L 2X Laemmli sample buffer.
9. Store the beads frozen for future analysis or boil the beads for 5 minutes.
10. Collect the beads after boiling using a microcentrifuge pulse.
11. Perform SDS-PAGE and western blot analysis on a sample of the supernatant fraction.

**Immunocytochemistry**

1. Plate approximately 200  $\mu$ L of cell suspension into each well of a slide. Incubate 24 hours in a 37°C CO<sub>2</sub> incubator.
2. Wash the cells three times for 10 minutes with PBS. Do not shake cells.
3. Add fix (4% paraformaldehyde) in PBS for 10 minutes at room temperature.
4. Wash the cells with PBS, twice, for 15 minutes. Do not shake.
5. Add 0.25% Triton X-100 in PBS for 5 minutes to permeabilize.
6. Wash the cells with PBS, twice, for 15 minutes. Do not shake.
7. Add 400  $\mu$ L of 1% BSA in PBS and incubate for 1 hour at room temperature.
8. Wash the cells with PBS for 15 minutes.
9. Incubate the cells with **1-5  $\mu$ g/mL anti- $\beta$ -Catenin** in 1% BSA in PBS and incubate overnight at 4°C.
10. Wash the cells twice with PBS for 15 minutes.
11. Incubate the cells in the dark with a 1:250 dilution of goat anti-rabbit IgG fluorescein conjugated secondary antibody in 1% BSA in PBS for 1 hour at room temperature.
12. Wash the cells three times with PBS for 15 minutes.
13. Examine the cells under a fluorescent microscope.

**Cell Fractionation**

1. Prepare a membrane-enriched fraction by collecting cells in TES resuspension buffer (20 mM Tris-base, pH 7.4, 1 mM EDTA, pH 8.0, 150 mM NaCl, and 0.25 M sucrose) and homogenize on ice with 30-40 cycles in a Dounce homogenizer.
2. Centrifuge the suspension for 10 minutes at 500 x g at 4°C.
3. Spin the resulting supernatant for 90 minutes at 100,000 x g at 4°C. The supernatant following this spin represents the cytosolic fraction.
4. Solubilize the membrane-enriched pellet from above for 1 hour in solubilization buffer (10 mM Tris-base, pH 8.0, 1 mM EDTA, 100 mM NaCl, 1 mM DTT, and 0.1% NP-40) and spin at 13,000 x g for 3 minutes at 4°C to remove debris. This supernatant contains the membrane-enriched fraction.

**RELATED PRODUCTS (specific)**

cat #	description
05-601	■ Anti-β-Catenin (non-phospho), clone 8E4
05-613	■ Anti-β-Catenin, clone 2H4A7
05-665	■ Anti-Active-β-Catenin (anti-ABC), clone 8E7
AB19022	■ Anti-beta-Catenin
MAB2081	■ Anti-beta-Catenin, clone 5H10
MAB3790	■ Anti-beta-Catenin, clone 9F12.2
12-537	■ β-Catenin, GST
M-003482	■ β-Catenin SMARTpool® siRNA reagent
60-014	■ β-Catenin siRNA/siAb™ Assay Kit
62-244	■ siRNA plasmid, pKD-β-Catenin-v4
46-626	■ Active β-Catenin Beadmates™
12-349	■ Goat Anti-Mouse IgG, HRP conjugate

**RELATED PRODUCTS (non-specific)**

cat #	description
IPVH00010	■ Immobilon-P 26.5 cm x 3.75 m Roll PVDF 0.45 um
IPFL00010	■ Immobilon-FL 26.5 cm x 3.75 m Roll PVDF 0.45 um
IPVH07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.45 mm (sheet) 50/pk
ISEQ00010	■ Immobilon-P SQ 26.5 cm x 3.75 m 1 roll PVDF 0.2 um
ISEQ07850	■ Immobilon-P 7 x 8.4 cm PVDF 0.2 mm (sheet) 50/pk
IPFL07810	■ Immobilon-FL 7 x 8.4 cm PVDF 0.45 mm (sheet) 10/pk
WBKLS0050	■ IMMOBILON WESTERN CHEMILUM HRP SUBSTRATE 50 mL
17-373SP	■ Spray & Glow™ ECL Western Blotting 40 mL
2060	■ Re-Blot Western Blot Recycling Kit
2500	■ Re-Blot Plus Western Blot Recycling Kit
B2080-175GM	■ Blot Quick Blocker Membrane Blocking Agent 175G
2170	■ CHEMIBLOCKER-1LT
20-200	■ IMMUNOBLOT BLOCKING REAGENT 20G
12-302	■ EGF-Stimulated A431 Cell Lysate
12-349	■ Goat Anti-Mouse IgG, HRP conjugate
12-110	■ Phosphotyrosine control (EGF-stim A431 cell lysate)

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