



cell signaling solutions

Certificate of Analysis

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Anti- β -Catenin (rabbit polyclonal IgG) Catalog # 06-734 Lot # 24042

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

Specificity: Recognizes β -Catenin, Mr 92kDa.

Species Cross-reactivity: Human, bovine, rat, rabbit and mouse.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the original vial prior to removing the cap.

Formulation: 200 μg of protein A purified rabbit IgG in 200 μl of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C .

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

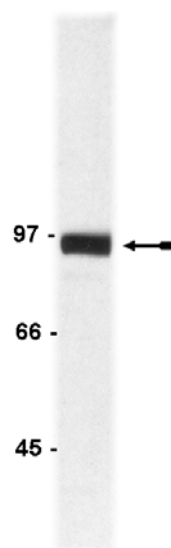
Quality Control Testing

Immunoblot Analysis: 0.1-2 $\mu\text{g}/\text{ml}$ of this lot detected β -catenin in an A431 total 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.

Immunoprecipitation: 4 μg of a previous lot immunoprecipitated β -catenin from 500 μg of A431 membrane fraction lysate.

Immunocytochemistry: 1-5 $\mu\text{g}/\text{ml}$ of this 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.



Immunoblot Analysis

Representative blot from a previous lot. A431 total cell lysate was resolved by electrophoresis, transferred to nitrocellulose, and probed with anti- β -Catenin (0.1 $\mu\text{g}/\text{ml}$). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates β -Catenin (92kDa).

General References:

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

Immunoblot Protocol

1. Lyse cells from a 150 mm culture plate directly in 1 ml of boiling Laemmli sample buffer containing 5% β -mercaptoethanol. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) 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 **0.1-2 μ g/ml of anti- β -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 Protocol

1. Dilute the membrane-enriched fraction (see protocol below) to 1mg/ml with PBS.
2. Add **4 μ g of anti- β -Catenin** to 500 μ g-1mg membrane-enriched fraction.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 μ l (50 μ l packed beads) of washed Protein A agarose bead slurry (Catalog # 16-125).
5. Gently rock the reaction mixture at 4°C for 2 hours.
6. 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.
7. Resuspend the agarose beads in 60 μ l 2X Laemmli sample buffer.
8. Store the beads frozen for future analysis or boil the beads for 5 minutes.
9. Collect the beads after boiling using a microcentrifuge pulse.
10. Perform SDS-PAGE and immunoblot analysis on a sample of the supernatant fraction.

Cell Fractionation Protocol

1. Prepare a membrane-enriched fraction by collecting cells in TES resuspension buffer (20mM Tris-base, pH 7.4, 1mM EDTA, pH 8.0, 150mM NaCl, and 0.25M 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 (10mM Tris-base, pH 8.0, 1mM EDTA, 100mM NaCl, 1mM 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.

Immunocytochemistry Protocol

1. Plate approximately 200 μ l of cell suspension into each well of a slide. Incubate 24 hours in a 37°C CO₂ 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 μ 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 μ g/ml anti- β -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.