

## Anti- $\beta$ -Catenin

(rabbit polyclonal IgG)

Catalog # 06-734

Lot # 17036

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

**Specificity:** Recognizes  $\beta$ -Catenin at 92kDa.

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

**Storage and Stability:** Stable for 2 years at  $-20^{\circ}\text{C}$  from date of shipment. For maximum recovery of product, centrifuge the original vial.

**Formulation:** **200mg** of protein A purified rabbit IgG in **200ml** of 0.07M Tris-glycine, pH 7.4, 0.105M NaCl, 0.035% sodium azide containing 30% glycerol. Liquid at  $-20^{\circ}\text{C}$ .

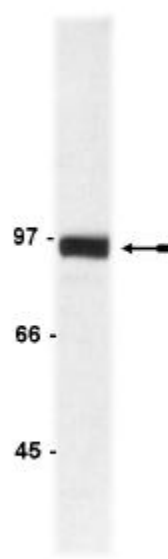
**FOR RESEARCH USE ONLY**  
**NOT FOR USE IN HUMANS**

## Quality Control Testing

**Immunoblot Analysis:** 0.1-1 $\mu\text{g/ml}$  of this lot detected  $\beta$ -catenin in an A431 total cell lysate.

**Immunoprecipitation:** 4 $\mu\text{g}$  of this lot immunoprecipitated  $\beta$ -catenin from 500 $\mu\text{g}$  of A431 membrane fraction lysate.

**Immunocytochemistry:** 1-5 $\mu\text{g/ml}$  of this lot showed positive immunostaining for  $\beta$ -catenin at cell-cell junctions of A431 cells fixed with 4% paraformaldehyde and permeabilized with 0.25% Triton X-100.



### Immunoblot Analysis

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

**Background:**  $\beta$ -catenin links cadherin cell adhesion molecules to the cytoskeleton has recently been implicated in the wnt/wingless signaling pathway and in the progression of neoplasia.  $\beta$ -catenin associates with the tumor suppressor adenomatous polyposis coli (APC) and functions as a co-activator of the TCF transcription factor family. It is regulated by glycogen synthase kinase (GSK) 3 $\beta$  that phosphorylates and targets  $\beta$ -catenin for ubiquitin-mediated proteolysis. Stabilization of  $\beta$ -catenin, either by wnt-mediated inhibition of GSK 3 $\beta$  or by mutation (as found in numerous colorectal tumors and melanoma cell lines), results in activation of TCF responsive genes which are thought to play key roles in development and cancer progression. A peptide corresponding to the GSK 3 $\beta$  phosphorylation site of  $\beta$ -catenin was used as an immunogen for this polyclonal antibody..

### 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%  $\beta$ -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 (PBS-MLK) for 20 minutes at 20-25°C with constant agitation.
3. Incubate the nitrocellulose with **0.1-1ng/ml of anti-b-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 a-rabbit** HRP conjugated IgG, 1:3000 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 **4ng of anti-b-Catenin** to 500 $\mu$ g-1mg membrane-enriched fraction.
3. Gently rock the reaction mixture at 4°C overnight.
4. Capture the immunocomplex by adding 100 $\mu$ l of washed Protein A agarose bead slurry (50 $\mu$ l packed beads).
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 50 $\mu$ l 2X Laemmli sample buffer.
8. The agarose beads can either be frozen for later use or suspended in Laemmli sample buffer and boiled for 5 minutes. Collect the beads by a microcentrifuge pulse. SDS-PAGE and subsequent immunoblot analysis can be performed on a sample of the supernatant.

### 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 $\mu$ 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 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.