

## Product Information

**Anti- $\beta$ -Catenin-Cy3 antibody, Mouse monoclonal**  
clone 15B8, Purified from hybridoma cell culture

Product Number **C7738**

### Product Description

Anti- $\beta$ -Catenin-Cy3 antibody, Mouse monoclonal (mouse IgG1 isotype) is derived from the 15B8 hybridoma, produced by fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with recombinant chicken catenin.<sup>1</sup> The conjugate is prepared by conjugation of the purified antibody isolated from ascites to Cy3\*, and the conjugate is purified by gel filtration to remove unbound Cy3 fluorophore.

Anti- $\beta$ -Catenin-Cy3 antibody, Mouse monoclonal specifically recognizes mammalian and avian  $\beta$ -catenin. Applications include direct immunofluorescence staining of a variety of acetone/methanol fixed cultured cells.

Cell adhesion is vitally important during development and in the adult organism is necessary for sorting of cells, induction of cellular morphogenesis, and maintenance of tissue integrity.<sup>1-3</sup>  $\text{Ca}^{2+}$ -dependent cell adhesion is mediated by a multifunctional family of transmembrane glycoproteins termed cadherins.<sup>3</sup> Cadherins are concentrated in cell-cell adherens junctions, where cells come into close contact with one another. Cadherins, self associate specifically via their extracellular domains. Studies supporting a role for cadherins in morphogenesis have led to the hypothesis that cadherins are crucial for segregation and sorting of different cells expressing different cadherin types. During recognition and adhesion between cells, cadherins regulate homophilic,  $\text{Ca}^{2+}$ -dependent interactions in cells. This initiates a cascade of events that leads to the structural and functional reorganization of cells, including formation of junctional complexes (tight junction, *Zonula adherens*, desmosomes), organization of the actin cytoskeleton at the apical junctional complex, assembly of the membrane cytoskeleton, and development of membrane domains. The mechanism of cadherin function involves both specific binding of extracellular domains at the cell surface and interactions with components of the cytoplasm.

Studies have identified three cytoplasmic proteins, known as  $\alpha$ -,  $\beta$ -, and  $\gamma$ -catenin, that bind noncovalently to the cytoplasmic domain of cadherins.<sup>4</sup>  $\beta$ -catenin (92-97 kDa), shares 70% sequence identity to a protein encoded by *Drosophila armadillo*, a segment polarity gene. Both *armadillo* and  $\beta$ -catenin share considerable homology with plakoglobin, which has been proposed to be  $\gamma$ -catenin. The homology between  $\beta$ -catenin and *armadillo* raised the possibility that  $\beta$ -catenin has a developmental signaling role in vertebrates. In addition to these roles in normal development and physiology,  $\beta$ -catenin is also a critical target in the development of a variety of human tumors.<sup>5-8</sup> Finally,  $\beta$ -catenin binds to a diverse set of other proteins, including presenilins, epidermal growth factor (EGF) receptors, actin-binding protein fascin, and the transcription factor Teashirt.<sup>9-10</sup>

$\beta$ -catenin protein is composed of a series of protein-protein interaction motifs that allow it to function as a scaffold. The N-terminus domain contains the binding site for  $\alpha$ -catenin as well as phosphorylation sites recognized by GSK3 $\beta$  and the C-terminus contains the transcriptional activation domain and binding site for Teashirt.<sup>9-10</sup>  $\beta$ -catenin translocates into the nucleus, where it complexes with transcription factors of the LEF-1 family and thus regulates the expression of specific genes. By playing a dual role, structural role in cell-cell junctions and regulatory role in the nucleus,  $\beta$ -catenin transduces changes in cell adhesion and junction formation to control transmembrane signaling and gene expression.<sup>1, 11</sup>  $\beta$ -catenin-mediated signaling depends on its accumulation and subsequent translocation into the nucleus. The level of  $\beta$ -catenin in the cell is regulated by its association with the tumor suppressor molecule adenomatous polyposis coli (APC), axin, and glycogen synthase kinase 3 $\beta$  (GSK3 $\beta$ ). In the APC-axin-GSK3 $\beta$  complex, brought together by axin, GSK3 $\beta$  phosphorylates  $\beta$ -catenin at multiple serine or threonine residues at the amino-terminal region of  $\beta$ -catenin,<sup>1</sup> thereby marking  $\beta$ -catenin for

degradation by the ubiquitin-proteasome pathway. The significance of  $\beta$ -catenin phosphorylation for its stability is most clearly manifested in several types of human cancers. The failure of  $\beta$ -catenin degradation in cells expressing mutant APC, leads to the accumulation of  $\beta$ -catenin and is common in human colon cancer and melanoma.<sup>11</sup> Moreover, a single amino acid mutation may occur at one of the four critical phosphoserine or threonine residues, at the  $\beta$ -catenin amino terminal region, in the consensus GSK3 $\beta$  phosphorylation site. These mutations result in deregulated accumulation of  $\beta$ -catenin and thereby, increased signaling through the TCF/ $\beta$ -catenin transcriptional complex contributing to tumorigenesis.

### Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin (BSA) and 15 mM sodium azide.

Antibody concentration: approx. 1 mg/ml

F/P Molar Ratio: 3–9

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

### Storage/Stability

Store at 2–8 °C. Working dilutions should be discarded if not used within 12 hours. Store the product protected from light.

### Product Profile

Direct immunofluorescence: a minimum working antibody dilution of 1:100 is recommended using methanol/acetone-fixed cultured bovine MDBK/MCF7 cells.

**Note:** In order to obtain the best results using various techniques and preparations, we recommend determining the optimal working dilution by titration.

### References

1. Ben-Ze'ev, A., and Geiger, B., *Curr. Opin. Cell Biol.*, **10**, 629-639 (1998).
2. Edelman, G., and Crossin, K., *Ann. Rev. Biochem.*, **60**, 155-190 (1991).
3. Takeichi, M., *Science*, **251**, 1451-1455 (1991).
4. Nagafuchi, A., and Takeichi, M., *EMBO J.*, **7**, 3679-3684 (1988).
5. Peifer, M., and Polakis, P., *Science*, **287**, 1606-1609 (2000).
6. Korinek, V., et al., *Science*, **275**, 1784-1787 (1997).
7. Morin, P.J., et al., *Science*, **275**, 1787-1790 (1997).
8. Rubinfeld, B., et al., *Science*, **275**, 1790-1792 (1997).
9. Zhurinsky, J., et al., *J. Cell Sci.*, **113**, 3127-3139 (2000).
10. Simcha, I., et al., *Mol. Biol. Cell*, **12**, 1177-1188 (2001).
11. Shtutman, M., et al., *Proc. Natl. Acad. Sci. USA*, **96**, 5522-5527 (1999).

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SG,KAA/RM,PHC,AD 07/20-1