3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Monoclonal Anti-phospho-β-Catenin (pSer^{33})
Clone BC-76
Purified Mouse Immunogobulin

Product Number **C 2363**

Product Description

Monoclonal Anti-phospho-β-Catenin (pSer^{33}) (mouse IgM isotype) is derived from the BC-76 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a synthetic phosphorylated peptide corresponding to amino acids 32-45 (pSer^{33}) of human β-Catenin. The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-Phospho-β-Catenin (pSer^{33}) recognizes human β-Catenin phosphorylated at Ser^{33}. The antibody may be used in ELISA and immunoblotting (approximately 94 kDa). Additional weaker bands may also be present.

Cell adhesion is important during development, as well as in sorting of cells, induction of cellular morphogenesis and maintenance of tissue integrity.[1-3] Ca^{2+}-dependent adhesion of cells involves a multifunctional family of transmembrane glycoproteins termed cadherins that regulate homophilic interactions in cells. These interactions initiate a cascade of events that leads to the structural and functional reorganizations of cells. Cadherins function is mediated by both specific binding of extracellular domains at the cell surface and interaction with components of the cytoplasm. These components include α-, β- and γ-catenin (plakoglobin) that bind to the cytoplasmic domain of cadherins.[4] β-catenin (92-97 kDa) shares 70% sequence identity to a protein encoded by Drosophila *armadillo*, a segment polarity gene.[5-8]

β-catenin binds to a diverse set of proteins including the presenilins, epidermal growth factor receptor (EGF-R), the actin-binding protein fascin and the transcription factor Teashirt.[9-10] β-catenin is composed of a series of protein-protein interaction motifs that allow it to function as a scaffold. The N-terminus domain, containing the binding site for α-catenin and the phosphorylation sites, is recognized by GSK3β. The C-terminus contains the transcriptional activation domain and the binding site for Teashirt.[9-10] β-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 such a dual function, i.e. a structural role in cell-cell junctions and a regulatory role in the nucleus, β-catenin can transduce changes in cell adhesion and junction formation to control transmembrane signaling and gene expression.[1, 11]

β-catenin-mediated signaling depends on its accumulation and subsequent translocation into the nucleus. The level of β-catenin is regulated by its association with the tumor suppressor molecule Adenomatous Polyposis Coli (APC), axin, and GSK3β. In this complex, GSK3β phosphorylates β-catenin at multiple serine or threonine residues present in the amino terminal region of β-catenin,[1] thereby marking β-catenin for degradation by the proteasome pathway. The significance of β-catenin phosphorylation for its stability is most clearly manifested in several types of human cancers. In cells expressing mutant APC, common in human colon cancer and melanoma, β-catenin accumulates due to the failure of its degradation.[1] Moreover, a single amino acid mutation at one of the four critical serine or threonine residues (Ser33, -37 and -45 and Thr-41) at the amino terminal region in the consensus GSK3β phosphorylation site, results in deregulated accumulation of β-catenin and thereby increased signaling through the TCF/β-catenin transcriptional complex, which contributes to tumorigenesis. Immunofluorescence studies indicate that phosphorylated β-catenin accumulates in the nuclei of Madin-Darby Canine Kidney (MDCK) and BCAP (human breast cancer) cells and is transiently associated with adherens junctions.[12] It was recently shown that phosphorylation of Ser^{45} and Thr^{41} precedes the phosphorylation of Ser^{33} and Ser^{37}. Phosphorylation is mediated by casein kinase Iα (CKIα), an axin associated kinase.[13]

Monoclonal antibodies reacting specifically with β-catenin phosphorylated at Ser^{33} are essential tools for defining the role of the phosphorylated form in the distributions, interactions, and regulation/deregulation of β-catenin in signal transduction.

Reagent
Monoclonal Anti-phospho-β-Catenin ($pSer^{33}$) is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide.
Antibody Concentration: approx. 1 mg/ml

Precautions and Disclaimer
Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability
For continuous use, store at 2-8 °C for up to one month. For prolonged storage, freeze in working aliquots at –20 °C. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is also not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilution samples should be discarded if not used within 12 hours.

Product Profile
A working concentration of 5 µg/ml is determined by immunoblotting, using a total cell extracts of cultured HEK-293T (human embryonal kidney) cells treated with the proteasome inhibitor Z-Leu-Leu-Leu-al (MG132) Product No. C 2211.

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

References

1. Ben-Ze'ev, A., and Geiger, B., Curr. Opin. Cell Biol., **10**, 629-639 (1998).
2. Edelman, G.M., and Crossin, K.L., Annu. 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).
12. Sadot, E., et al., J. Cell Sci., **115**, 2771-2780 (2002).
13. Liu, C., et al., Cell, **108**, 837-847 (2002).

EK/KAA 01/03