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# Product Information

**Anti-phospho-β-Catenin (pSer$^{33}$/pSer$^{37}$) antibody, Mouse monoclonal**
**clone BC-22, purified from hybridoma cell culture**

Product Number **C4231**

## Product Description

Anti-phospho-β-Catenin (pSer$^{33}$/pSer$^{37}$) antibody, Mouse monoclonal (mouse IgG2b isotype) is derived from the BC-22 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mouse immunized with a synthetic peptide corresponding to amino acids 32-45 (pSer$^{33, 37}$) of human β-catenin, conjugated to KLH. The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Product Number ISO2.

Anti-phospho-β-Catenin (pSer$^{33}$/pSer$^{37}$) antibody, Mouse monoclonal reacts specifically with β-catenin phosphorylated at Ser$^{37}$, and Ser$^{33}$.[1] At the peptide level, the antibody is inhibited more efficiently by the pSer$^{37}$ peptide than by the pSer$^{33}$ peptide and is not inhibited by the nonphosphorylated peptide. The antibody does not detect the unphosphorylated or the Ser$^{33}$ phosphorylated protein. It does not recognize phosphorylated plakoglobin despite the high homology in the phosphorylation site with β-catenin.[1] The antibody may be used in various applications including ELISA, immunofluorscence,[1] and immunoblotting (approximately 94 kDa, and possibly also weaker bands at approximately 45 and 55 kDa, in cells treated with the proteasome inhibitor MG132).[1, 2] Reactivity has been observed with human, rat, mouse, and chicken β-catenin.

Cell adhesion is vitally important during development and in the adult organism. It is necessary in sorting of cells, induction of cellular morphogenesis, and maintenance of tissue integrity.[3-5] $Ca^{+2}$-dependent cell adhesion is mediated by a multifunctional family of transmembrane glycoproteins termed cadherins.[3] Cadherins are concentrated in cell-cell adherens junctions, where cells come into close contact with one another. Studies supporting a role for cadherins in morphogenesis have led to the hypothesis that cadherins are crucial for segregation and sorting out from one another, of different cells expressing different cadherin types.

During recognition and adhesion between cells, cadherins regulate homophilic, $Ca^{+2}$-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 α-, β-, and γ-catenin, that bind noncovalently 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. Both *armadillo* and β-catenin share considerable homology with plakoglobin, which has been proposed to be γ-catenin. The homology between β-catenin and *armadillo* raised the possibility that β-catenin has a developmental signaling role in vertebrates. In addition to these roles in normal development and physiology, β-catenin is also a critical target in the development of a variety of human tumors.[5-8] Finally, β-catenin binds to a diverse set of other proteins, including the presenilins, the epidermal growth factor (EGF) receptor, the actin-binding protein fascin, and the transcription factor Teashirt.[9, 10]

β-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 α-catenin, as well as phosphorylation sites recognized by GSK3β, whereas the C-terminus contains the transcriptional activation domain and the binding site for Teashirt.[9, 10] β-catenin can translocate into the nucleus, where it can complex with transcription factors of the LEF-1 family and thus regulate the expression of specific genes.

By playing dual structural roles in cell-cell junctions and in the regulation of 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 in the cell is regulated by its association with the tumor suppressor molecule adenomatous polyposis coli (APC), axin, and glycogen synthase kinase 3β (GSK3β). In the APC-axin-GSK3β complex, brought together by axin, GSK3β phosphorylates β-catenin at multiple serine or threonine residues at the amino terminal region of β-catenin,[1] thereby, marking β-catenin for degradation by the ubiquitin-proteasome pathway. The importance of β-catenin phosphorylation to its stability is most clearly manifested in several types of human cancers. The failure of β-catenin degradation in cells expressing mutant APC leads to the accumulation of β-catenin and is common in human colon cancer and melanoma.[11] Moreover, a single amino acid mutation may occur at one of the four critical phosphoserine or threonine residues (serine-33, -37, and -45, and threonine-41), at the β-catenin amino-terminal region, in the consensus GSK3β phosphorylation site. These mutations result in deregulated accumulation of β-catenin and, thereby, increased signaling through the TCF/β-catenin transcriptional complex, contributing to tumorigenesis.

Monoclonal antibodies reacting specifically with β-catenin phosphorylated at serine-33 and serine-37 are an essential tool in defining the interactions, distribution, and regulation/ deregulation of β-catenin and its role in signal transduction.

**Reagent**
Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody Concentration: ~2 mg/ml

**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**
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, or storage in "frost-free" freezers, is 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**
Immunoblotting: a working antibody concentration of 5-10 μg/ml is recommended using a whole extract of cultured 293 (human embryonal kidney) cells treated with the proteasome inhibitor MG132.

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

**References**

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