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Product Information

Anti-α-Catenin
produced in rabbit, delipidized, whole antiserum

Catalog Number **C2081**

Product Description

Anti-α-Catenin is produced in rabbit using a synthetic peptide (His-Val-Asn-Pro-Val-Gln-Ala-Leu-Ser-Glu-Phe-Lys) conjugated to KLH as immunogen. The peptide corresponds to amino acids 890-901 of human/mouse α-catenin. The antiserum has been treated to remove lipoproteins.

Anti-α-Catenin may be used for the immuno-localization of α-catenin by various immunohistochemical methods using frozen tissue sections and cultured cells. It may be used to detect α-catenin by other assays including dot blot immunoassay and immunoblotting.

The distinct peripheral cytosolic proteins, α-, β- and γ-catenin (102 kDa, 94 kDa and 86 kDa, respectively) are found in varying abundance in many developing and adult tissues.[1,2,3] The catenins bind, directly or indirectly, to the conserved cytoplasmic tail domain of the cell-adhesion cadherins.

Cadherins are transmembrane cell surface glycoprotein molecules that mediate calcium-dependent intercellular interactions and are important for tissue morphogenesis.[4] The linkage of the epithelial E-cadherin/uvomorulin to actin is essential for the cell binding function of this cadherin. Catenins link E-cadherin to other integral membrane proteins such as Na^+/K^+-ATPase, or to cytoplasmic proteins such as fodrin, ankyrin, Src and Yes kinases[5] and are modulated by Wnt-1 protooncogene.[6,7] They are considered good candidates for mediating transduction of cell-cell contact positional signals to the cell interior.[4,5] Within its conserved regions, α-catenin shows 30% identity to vinculin, a protein found mainly in focal cell-cell and cell substrate adhesions.[2,3] Vinculin is known to interact with α-actinin, which in turn is associated with actin filaments in their site of attachment to the cell membrane focal contacts. α-Catenin appears to be capable of interacting with N-cadherin and P-cadherin. Absence of α-catenin is found in certain tumor cell lines.[8] Frequent reduction of α-catenin levels in human carcinomas of the esophagus, stomach and colon is also reported.[9]

Enhancement of tumor cell invasion and metastatic ability of such cells following catenins down-regulation is speculated. Prostate cancer development appears to be correlated with α-catenin gene deletions. β-Catenin and plakoglobin (probably identical to γ-catenin) are structural and functional mammalian homologues of armadillo, a *Drosophila* protein involved in signal transduction. β-Catenin binds directly to the cytoplasmic tail of E-cadherin. It seems to bind to the amino terminus of α-catenin and interacts with the cytosolic protein product of the human tumor suppressor gene APC.[10] Mutations in this gene occur early in colon carcinogenesis.

Such mutations are linked to familial adenomatous polyposis and to progression of sporadic colorectal and gastric tumors. The preferential interaction of β-catenin with the APC protein involves a 15-amino acid repeat in the latter[11] and β-catenin cell levels seem to be controlled by APC.[12] The central core region of β-catenin is involved in mediation of the interaction of cadherin-catenin complex with the epidermal growth factor receptor.[13]

β-Catenin is the target of two signal transduction pathways mediated by the protooncogenes Src and Wnt-1. The protein, $p120^{cas}$, which exhibits structural similarity to β-catenin and plakoglobin, may represent another catenin associated with cadherin.[14] Polyclonal antibodies to type-specific catenin peptides are useful tools in the study of these proteins. Such antibodies recognize the respective catenin type in a variety of immunological techniques such as immuno-precipitation, immunoblotting, immunofluorescence and immunoperoxidase in different cell types from various species.

Reagent

Supplied as a liquid containing 15mM sodium azide as preservative.

Protein Concentration: 40-100 mg/ml by Biuret

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

Storage and Stability
For continuous use, store at 2-8 °C for up to one month. For extended storage freeze in working aliquots. 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.

Specificity
Anti-α-Catenin reacts in dot blot immunoassay with α-catenin peptide amino acids 890-901 conjugated to BSA. It reacts with a 102 kDa protein in extracts of Madin-Darby Bovine Kidney (MDBK) cultured cell line using immunoblotting. The antiserum shows no cross-reactivity with β-catenin peptide (amino acids 768-781) conjugated to BSA. Specific staining in immunoblotting is inhibited following preincubation of the diluted antiserum with the α-catenin peptide. The antibody stains α-catenin in frozen sections and cultured MDBK epithelial cells.

Product profile
Indirect Immunohistology: a minimum working dilution of 1:2,000 was determined using bovine kidney frozen sections.
Indirect Immunofluorescence: a minimum working dilution of 1:2,000 was determined using cultured MDBK cells.
Indirect Immunoblotting: a minimum working dilution of 1:4,000 was determined using cultured MDBK cells extract.

Note: In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

References

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Indirect Immunofluorescence Staining of Cultured Cells with Anti-α-Catenin

Materials:

1. Cultured cells grown to confluence on coverslips or chamber slides.
2. Solution A: PBS containing 1 mM $MgCl_2$ and 0.1 mM $CaCl_2$.
3. Solution B: Methanol, cooled to –20 °C.
4. Solution C: Acetone, cooled to –20 °C.
5. Anti-α-Catenin
6. Anti-Rabbit IgG-FITC, Catalog Number F0511.
7. Aqueous mounting medium.
8. Cover glasses (24 x 50 mm)/chamber slides.
9. Fluorescence microscope.

Indirect Immunofluorescent staining:

1. Remove cover glasses or chamber slides with cells from incubator. Discard medium.
2. Rinse with solution A. Drain excess solution.
3. Fix in solution B for 10 minutes at –20 °C.
4. Drain excess solution B and fix in solution C for 1 minute at –20 °C.
5. Rinse three times (5 minutes each) with solution A.
6. Dilute Anti-α-Catenin in solution A to dilution.
7. Apply antibody to slide and incubate for 2 hours at room temperature in a humid chamber.
8. Rinse as in step 5.
9. Dilute Anti-Rabbit IgG-FITC in Solution A to appropriate dilution.
10. Apply antibody to slide and incubate for 30 minutes at room temperature in a humid chamber.
11. Rinse as in step 5 and drain excess solution.
12. Mount and cover with cover glass.
13. Examine under fluorescence microscope.

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