abcam

Product datasheet

Anti-beta Catenin antibody [EP690Y] ab68183

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-beta Catenin antibody [EP690Y]
Description	Rabbit monoclonal [EP690Y] to beta Catenin
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human beta Catenin aa 650-750 (C terminal). The exact sequence is proprietary. Database link: **P35222**
Positive control	WB: HAP1, HeLa, A431, NIH/3T3 and C6 cell lysate. ICC/IF: Wild-type HAP1 cells. Flow Cyt (intra): A431 cells.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 40% Glycerol (glycerin, glycerine), 59% PBS
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP690Y

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab68183 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/20. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
WB	★★★★☆ **(1)**	1/500 - 1/1000. Detects a band of approximately 86 kDa (predicted molecular weight: 86 kDa).
ICC/IF		1/100 - 1/250.

Target

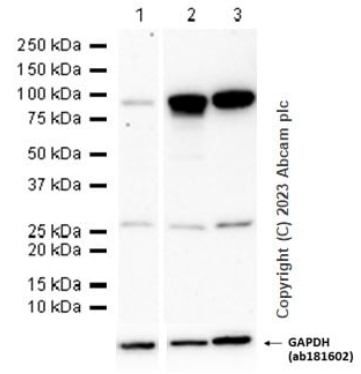
Function Key dowstream component of the canonical Wnt signaling pathway. In the absence of Wnt, forms a complex with AXIN1, AXIN2, APC, CSNK1A1 and GSK3B that promotes phosphorylation on N-terminal Ser and Thr residues and ubiquitination of CTNNB1 via BTRC and its subsequent degradation by the proteasome. In the presence of Wnt ligand, CTNNB1 is not ubiquitinated and accumulates in the nucleus, where it acts as a coactivator for transcription factors of the TCF/LEF family, leading to activate Wnt responsive genes.
Involved in the regulation of cell adhesion. The majority of beta-catenin is localized to the cell membrane and is part of E-cadherin/catenin adhesion complexes which are proposed to couple cadherins to the actin cytoskeleton.

Tissue specificity Expressed in several hair follicle cell types: basal and peripheral matrix cells, and cells of the outer and inner root sheaths. Expressed in colon.

Involvement in disease Defects in CTNNB1 are associated with colorectal cancer (CRC) [MIM:114500].
Note=Activating mutations in CTNNB1 have oncogenic activity resulting in tumor development. Somatic mutations are found in various tumor types, including colon cancers, ovarian and prostate carcinomas, hepatoblastoma (HB), hepatocellular carcinoma (HCC). HBs are malignant embryonal tumors mainly affecting young children in the first three years of life.
Defects in CTNNB1 are a cause of pilomatrixoma (PTR) [MIM:132600]; a common benign skin tumor.
Defects in CTNNB1 are a cause of medulloblastoma (MDB) [MIM:155255]. MDB is a malignant, invasive embryonal tumor of the cerebellum with a preferential manifestation in children.
Defects in CTNNB1 are a cause of susceptibility to ovarian cancer (OC) [MIM:167000]. Ovarian cancer common malignancy originating from ovarian tissue. Although many histologic types of ovarian neoplasms have been described, epithelial ovarian carcinoma is the most common form. Ovarian cancers are often asymptomatic and the recognized signs and symptoms, even of late-stage disease, are vague. Consequently, most patients are diagnosed with advanced disease.
Note=A chromosomal aberration involving CTNNB1 is found in salivary gland pleiomorphic adenomas, the most common benign epithelial tumors of the salivary gland. Translocation t(3;8)(p21;q12) with PLAG1.

Sequence similarities	Belongs to the beta-catenin family. Contains 12 ARM repeats.
Post-translational modifications	Phosphorylation by GSK3B requires prior phosphorylation of Ser-45 by another kinase. Phosphorylation proceeds then from Thr-41 to Ser-37 and Ser-33. EGF stimulates tyrosine phosphorylation. Phosphorylation on Tyr-654 decreases CDH1 binding and enhances TBP binding. Ubiquitinated by the SCF(BTRC) E3 ligase complex when phosphorylated by GSK3B, leading to its degradation. Ubiquitinated by a E3 ubiquitin ligase complex containing UBE2D1, SIAH1, CACYBP/SIP, SKP1, APC and TBL1X, leading to its subsequent proteasomal degradation.
Cellular localization	Cytoplasm. Nucleus. Cytoplasm > cytoskeleton. Cell junction > adherens junction. Cell junction. Cell membrane. Cytoplasmic when it is unstabilized (high level of phosphorylation) or bound to CDH1. Translocates to the nucleus when it is stabilized (low level of phosphorylation). Interaction with GLIS2 and MUC1 promotes nuclear translocation. Interaction with EMD inhibits nuclear localization.

Images



Western blot - Anti-beta Catenin antibody [EP690Y] (ab68183)

All lanes : Anti-beta Catenin antibody [EP690Y] (ab68183) at 1/1000 dilution

Lane 1 : RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate
Lane 3 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

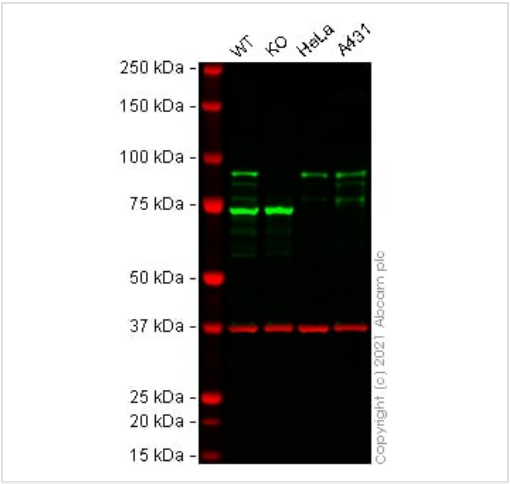
Predicted band size: 86 kDa
Observed band size: 90 kDa

Exposure time: 180 seconds

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

ab181602 was used as a GAPDH loading control.

Raw264.7 expresses low level of beta Catenin and stimulation is required to allow detection of the beta Catenin protein in this cell line, as described in PMID: 22983902 and PMID: 29137395.



Western blot - Anti-beta Catenin antibody [EP690Y] (ab68183)

All lanes : Anti-beta Catenin antibody [EP690Y] (ab68183) at 1/500 dilution

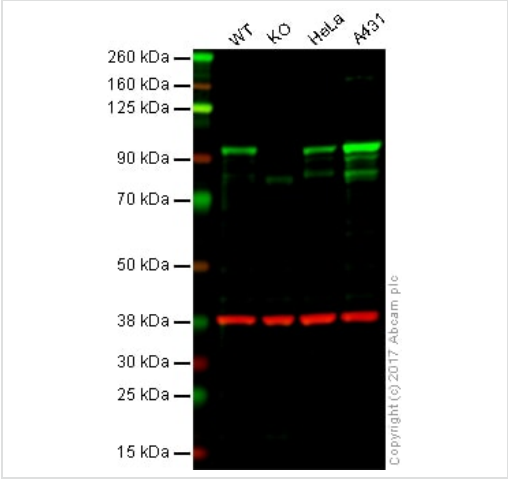
Lane 1 : Wild-type HepG2 cell lysate
Lane 2 : CTNNB1 knockout HepG2 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : A431 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 86 kDa
Observed band size: 85 kDa

False colour image of Western blot: Anti-beta Catenin antibody [EP690Y] staining at 1/500 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab68183 was shown to bind specifically to beta Catenin. A band was observed at 85 kDa in wild-type HepG2 cell lysates with no signal observed at this size in CTNNB1 knockout cell line. To generate this image, wild-type and CTNNB1 knockout HepG2 cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



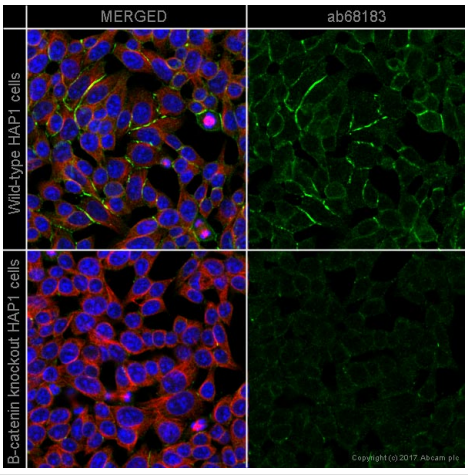
Western blot - Anti-beta Catenin antibody [EP690Y] (ab68183)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: CTNNB1 (β-Catenin) knockout HAP1 whole cell lysate (20 µg)
Lane 3: HeLa whole cell lysate (20 µg)
Lane 4: A431 whole cell lysate (20 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab68183 observed at 85 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab68183 was shown to specifically react with CTNNB1 (β-Catenin) in wild-type HAP1 cells along with additional cross reactive bands. No band was observed when knockout samples were used. Wild-type and CTNNB1 (β-Catenin) knockout samples were subjected to SDS-PAGE. ab68183 and **ab8245** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1/500 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

This image was generated using un-purified format of the antibody.

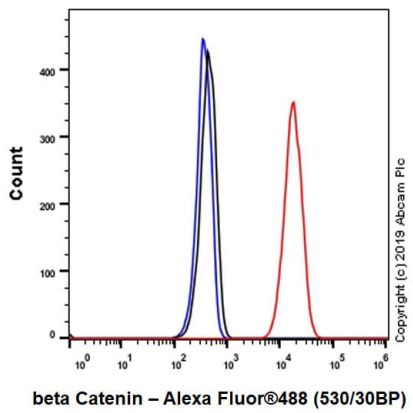


Immunocytochemistry/ Immunofluorescence - Anti-beta Catenin antibody [EP690Y] (ab68183)

ab68183 staining β-catenin in CTNNB1 (β-Catenin) wild-type HAP1 cells (top panel) and CTNNB1 (β-Catenin) knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab68183 at 1/250 dilution and **ab195889** at 1/250 dilution (shown in pseudocolour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

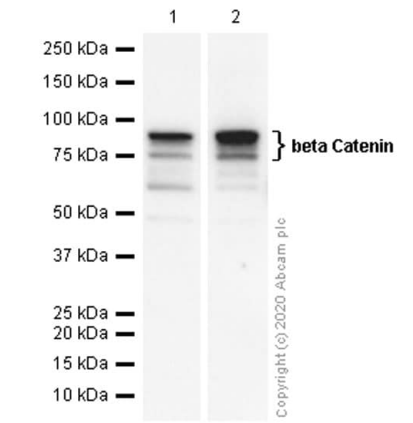
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This image was generated using un-purified format of the antibody.



Flow Cytometry (Intracellular) - Anti-beta Catenin antibody [EP690Y] (ab68183)

Intracellular Flow Cytometry analysis of A431 (Human epidermoid carcinoma epithelial cell) cells labeling beta Catenin with Purified ab68183 at 1/20 dilution (5 µg/mL) (Red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Western blot - Anti-beta Catenin antibody [EP690Y] (ab68183)

All lanes : Anti-beta Catenin antibody [EP690Y] (ab68183) at 1/1000 dilution (Purified)

Lane 1 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates
Lane 2 : C6 (Rat glial tumor glial cell) whole cell lysates

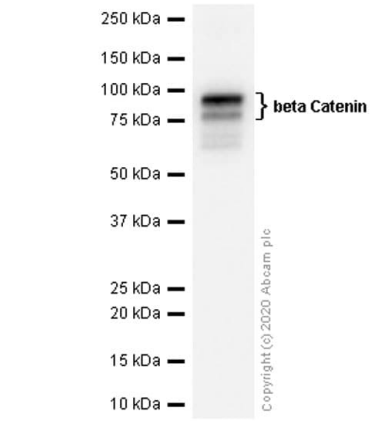
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 86 kDa
Observed band size: 75,90 kDa

Blocking/Diluting buffer: 5% NFDM/TBST.

Full-length beta catenin: 90kDa ; C-terminal cleavage fragment: 75kDa (PMID: 15492240).



Western blot - Anti-beta Catenin antibody [EP690Y] (ab68183)

Anti-beta Catenin antibody [EP690Y] (ab68183) at 1/1000 dilution (Purified) + A431 (Human epidermoid carcinoma epithelial cell) whole cell lysates at 15 µg

Secondary

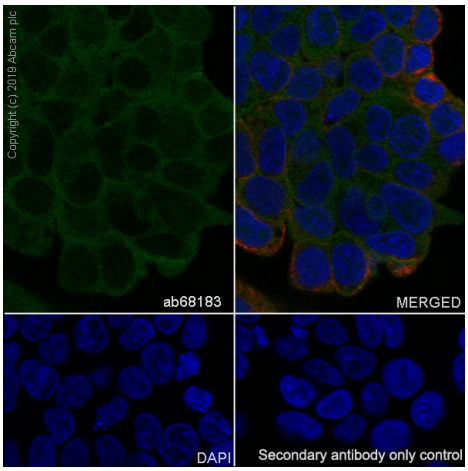
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 86 kDa

Observed band size: 75,90 kDa

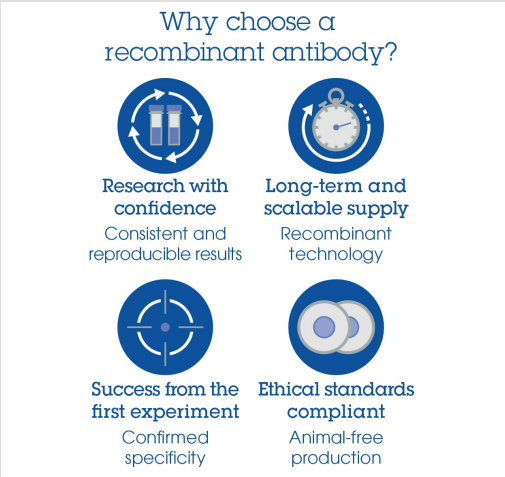
Blocking/Diluting buffer: 5% NFDM/TBST.

Full-length beta catenin: 90kDa ; C-terminal cleavage fragment: 75kDa (PMID: 15492240).



Immunocytochemistry/ Immunofluorescence - Anti-beta Catenin antibody [EP690Y] (ab68183)

Immunocytochemistry/ Immunofluorescence analysis of parental HAP1 (Wildtype control Human chronic myelogenous leukemia near-haploid cell line) cells labeling beta Catenin with Purified ab68183 at 1/100 dilution (10 µg/mL). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/mL). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 µg/mL) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Anti-beta Catenin antibody [EP690Y] (ab68183)

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