abcam

Product datasheet

Anti-beta Catenin antibody [IGX4794R-3] ab223075

KO VALIDATED Recombinant

2 References 6 Images

Overview

Product name	Anti-beta Catenin antibody [IGX4794R-3]
Description	Rabbit monoclonal [IGX4794R-3] to beta Catenin
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, IHC-P
Species reactivity	**Reacts with:** Mouse, Human
Immunogen	Full length protein corresponding to Mouse beta Catenin. Database link: Q02248
Positive control	WB: A549, Caco2, Hues7, NIH3T3, mES, E14Tg2a and wildtype HAP1 whole cell and human colon tissue lysates. IHC-P: FFPE Human Colon (Normal) and Human Colon Adenocarcinoma tissue sections. ICC/IF: MCF-7 and wildtype HAP1 cells.
General notes	This product was made using synthetic libraries and phage display technology. This antibody is a recombinant chimeric antibody. Rabbit chimeric monoclonal antibody (Human Fab/ Rabbit Fc).

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	Preservative: 0.02% Sodium azide Constituents: 1% BSA, PBS
Purity	Immunogen affinity purified
Clonality	Monoclonal
Clone number	IGX4794R-3
Isotype	IgG1

Applications

Our Abpromise guarantee covers the use of **ab223075** in the following tested applications.

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

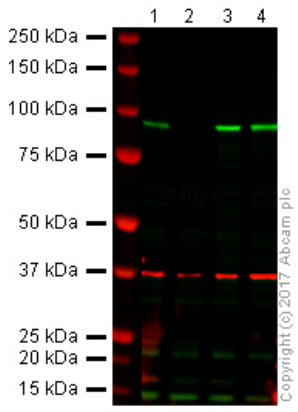
Application	Abreviews	Notes
WB		Use a concentration of 1 µg/ml. Detects a band of approximately 92 kDa (predicted molecular weight: 85 kDa).
ICC/IF		Use a concentration of 0.05 µg/ml.
IHC-P		Use a concentration of 0.25 µg/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

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 [IGX4794R-3] (ab223075)

Lane 1: Wild type HAP1 whole cell lysate (10 µg)

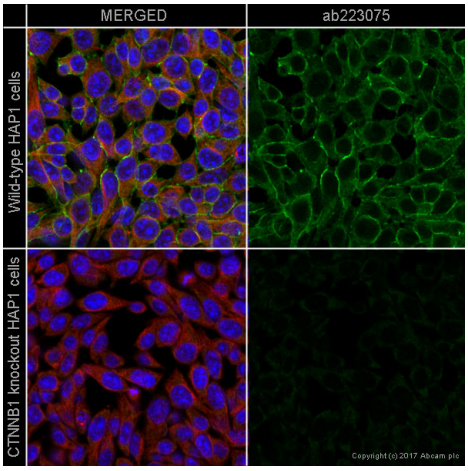
Lane 2: CTNNB1 (β-Catenin) knockout HAP1 whole cell lysate (10 µg)

Lane 3: A431 whole cell lysate (10 µg)

Lane 4: Caco-2 whole cell lysate (10 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab223075 observed at 95 kDa. Red - loading control, ab9484, observed at 37 kDa.

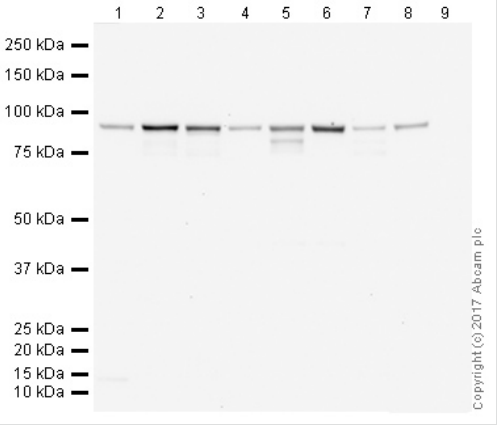
ab223075 was shown to specifically react with CTNNB1 (β-Catenin) in wild-type HAP1 cells along with additional cross reactive bands. No band was observed when knockout samples were used. Wild-type and CTNNB1 (β-Catenin) knockout samples were subjected to SDS-PAGE. ab223075 and ab9484 (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1 µg/ml 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.



Immunocytochemistry/ Immunofluorescence - Anti-beta Catenin antibody [IGX4794R-3] (ab223075)

ab223075 staining β-Catenin in 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 ab223075 at 0.05µg/ml and ab195889 at 1/250 dilution (shown in 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).



Western blot - Anti-beta Catenin antibody [IGX4794R-3] (ab223075)

All lanes : Anti-beta Catenin antibody [IGX4794R-3] (ab223075) at 1 µg/ml

Lane 1 : A549 (Human lung adenocarcinoma epithelial cell line) Whole Cell Lysate
Lane 2 : Caco 2 (Human colonic carcinoma cell line) Whole Cell Lysate
Lane 3 : HUES7 (Human embryonic stem cell line) Whole Cell Lysate
Lane 4 : NIH 3T3 (Mouse embryonic fibroblast cell line) Whole Cell Lysate
Lane 5 : mES (Mouse embryonic stem cell) Whole Cell Lysate
Lane 6 : E14Tg2a (Mouse embryonic stem cell line) Whole Cell Lysate
Lane 7 : Colon (Human) Tissue Lysate - adult normal tissue
Lane 8 : Wild type HAP1 whole cell lysate
Lane 9 : Beta Catenin knockout HAP1 whole cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Peroxidase AffiniPure Goat Anti-Rabbit IgG (H+L) at 1/50000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

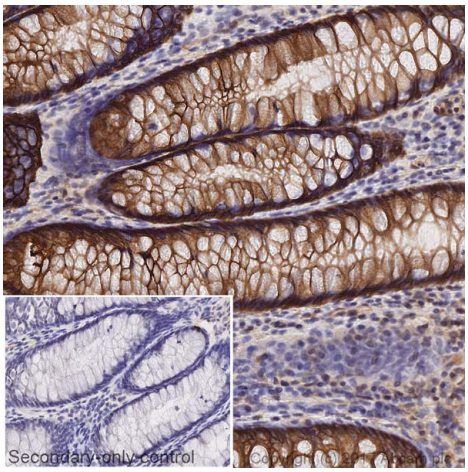
Predicted band size: 85 kDa

Observed band size: 92 kDa

why is the actual band size different from the predicted?

Exposure time: 3 minutes

This blot was produced using a 4-12% Bis-tris gel under the MOPS buffer system. The gel was run at 200V for 50 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% milk before being incubated with ab223075 overnight at 4°C. Antibody binding was detected using an anti-rabbit antibody conjugated to HRP, and visualised using ECL development solution ab133406.

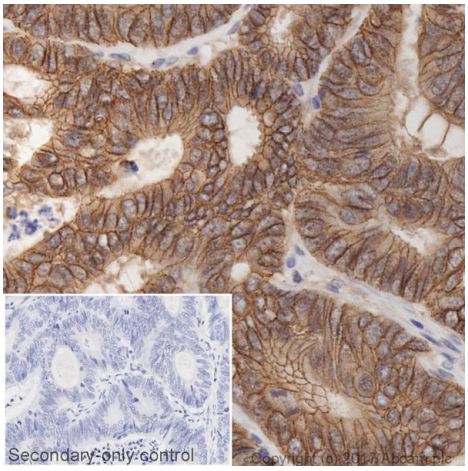


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Catenin antibody [IGX4794R-3] (ab223075)

IHC image of CTNNB1 staining in a section of formalin-fixed paraffin-embedded human colon (normal)*, performed on a Leica Bond™. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins, before blocking of endogenous biotin using ab64212. The section was then incubated with ab223075, 0.25ug/ml, for 15 mins at room temperature and detected using an HRP conjugated ABC system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset negative control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

**Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre*

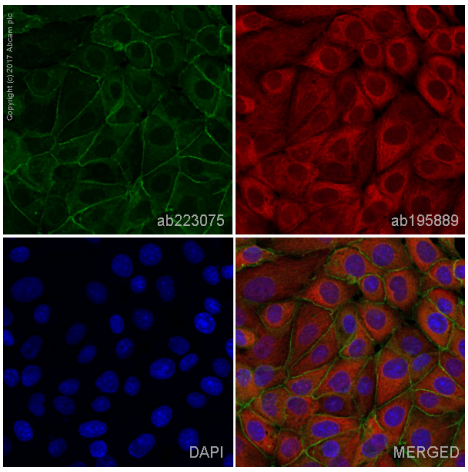


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Catenin antibody [IGX4794R-3] (ab223075)

IHC image of CTNNB1 staining in a section of formalin-fixed paraffin-embedded human colon adenocarcinoma*, performed on a Leica BOND™. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab223075, 0.1ug/ml dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset negative control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

**Tissue obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre*



Immunocytochemistry/ Immunofluorescence - Anti-beta Catenin antibody [IGX4794R-3] (ab223075)

ab223075 staining CTNNB1 in MCF-7 cells. The cells were fixed with 100% methanol (5 min), blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated overnight at +4°C with ab223075 at a 0.05µg/ml concentration, then detected with an Alexa Fluor® 488 goat anti-rabbit secondary antibody (ab150081) at a 1/1000 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue), and ab195889, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at a 1/250 dilution (shown in red).

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

This product also gave a positive signal in 4% formaldehyde (10 min) fixed MCF-7 cells under the same testing conditions.

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