

Product datasheet

Anti-beta Catenin antibody [IGX4794R-3] ab223075

KO **VALIDATED** Recombinant

★★★★★ [1 Abreviews](#) [18 References](#) [9 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-Fr, IHC-P |
| Species reactivity | Reacts with: Mouse, Human |
| Immunogen | Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers. |
| Positive control | WB: HCT116, 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 <u>synthetic libraries and phage display technology</u> . 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 | pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 0.05% BSA, 59% PBS, 40% Glycerol (glycerin, glycerine) |
| Clonality | Monoclonal |
| Clone number | IGX4794R-3 |
| Isotype | IgG |

Applications

The **Abpromise guarantee** 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-Fr | ★★★★★ (1) | Use at an assay dependent concentration. |
| 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 downstream 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 pilomatixoma (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

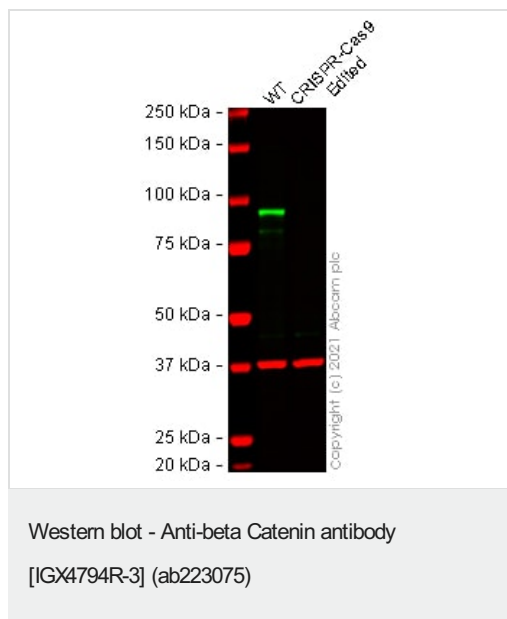
and enhances TBP binding.

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



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

Lane 1 : Wild-type HCT 116 cell lysate

Lane 2 : CTNNB1 CRISPR-Cas9 edited HCT 116 cell lysate

Lysates/proteins at 20 µg per lane.

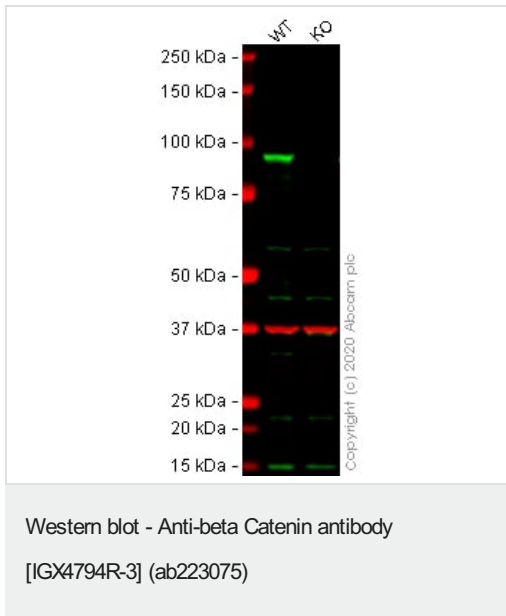
Performed under reducing conditions.

Predicted band size: 85 kDa

Observed band size: 95 kDa

False colour image of Western blot: Anti-beta Catenin antibody [IGX4794R-3] staining at 1 µg/ml, shown in green; Mouse anti-GAPDH antibody [6C5] ([ab8245](#)) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab223075 was shown to bind specifically to beta Catenin. A band was observed at 95 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in CTNNB1 CRISPR-Cas9 edited cell line [ab273712](#) (CRISPR-Cas9 edited cell lysate [ab275247](#)). The band observed in the CRISPR-Cas9 edited lysate lane below 95 kDa is likely to represent a truncated form of beta Catenin. This has not been investigated further and the functional properties of the gene product have not been determined. To generate this image, wild-type and CTNNB1 CRISPR-Cas9 edited HCT 116 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.



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

Lane 1 : Wild-type HCT116 cell lysate

Lane 2 : CTNNB1 knockout HCT116 cell lysate

Lysates/proteins at 20 µg per lane.

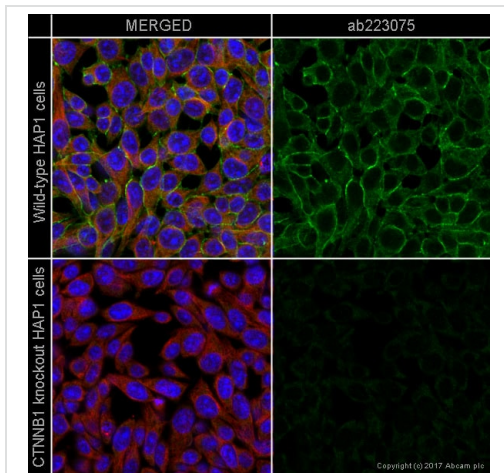
Performed under reducing conditions.

Predicted band size: 85 kDa

Observed band size: 95 kDa

Lanes 1 - 2: Merged signal (red and green). Green - ab223075 observed at 95 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

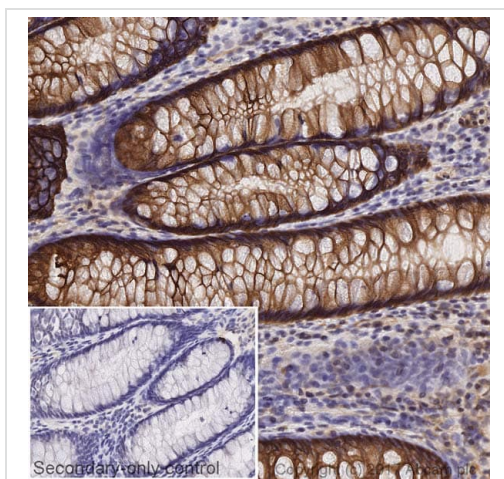
ab223075 was shown to react with Anti-beta Catenin in HCT 116 wild-type cells in western blot with loss of signal observed in CTNNB1 knockout cell line **ab273712** (CTNNB1 knockout cell lysate **ab275247**). HCT 116 wild-type and CTNNB1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in fluorescent western blot (TBS-based) blocking solution 50% (v/v) in TBS-T (0.1% Tween®) before incubation with ab223075 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at 1 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated 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 in 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 0.05 μ g/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 μ g/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

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

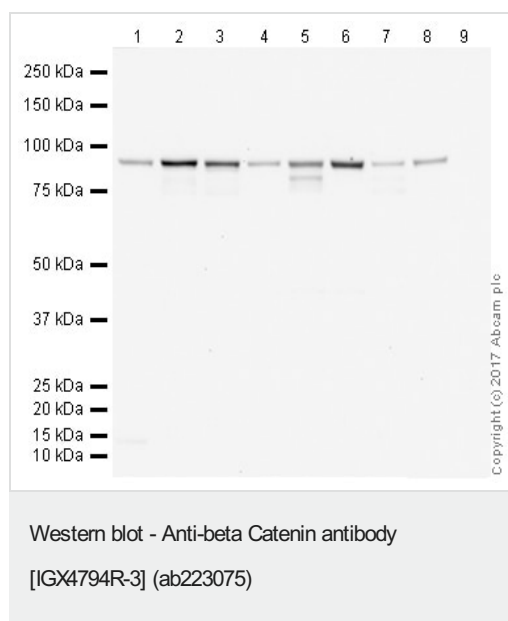


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Catenin antibody [IGX4794R-3] (ab223075)

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, 0.25 μ g/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*



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.

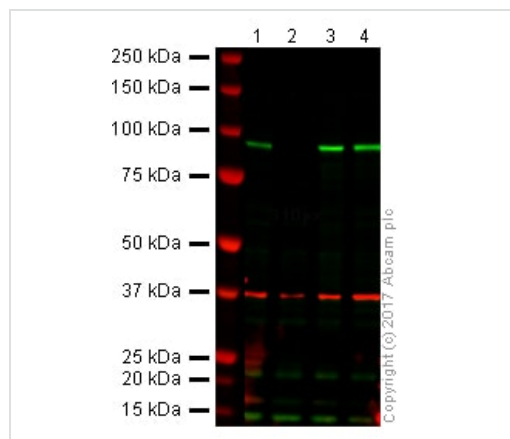
Predicted band size: 85 kDa

Observed band size: 92 kDa

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](#).



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

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

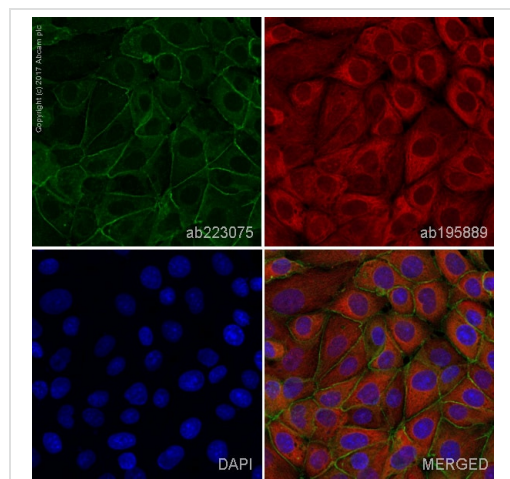
Lane 2: CTNNB1 (β-Catenin) knockout HAP1 whole cell lysate (10 µg)

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.

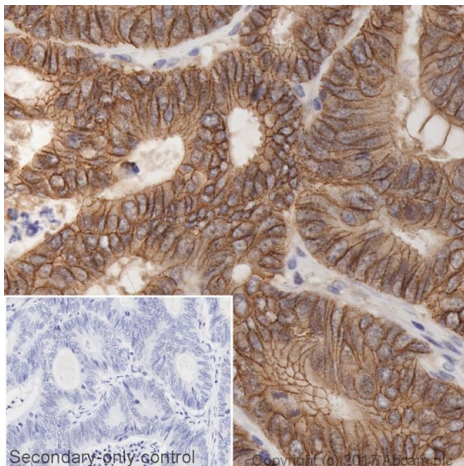


Immunocytochemistry/ Immunofluorescence - Anti-beta Catenin antibody [IGX4794R-3] (ab223075)

ab223075 staining 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.



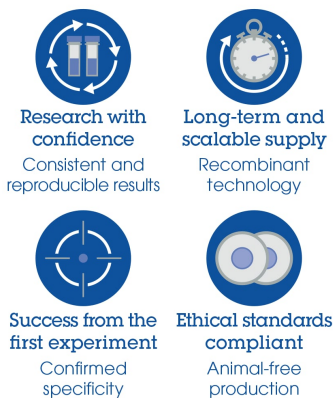
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Catenin antibody [IGX4794R-3] (ab223075)

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.

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Why choose a recombinant antibody?



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

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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