

Product datasheet

Anti-beta Catenin antibody [12F7] ab22656

KO VALIDATED

★★★★☆ 4 Abreviews 71 References 6 Images

Overview

Product name	Anti-beta Catenin antibody [12F7]
Description	Mouse monoclonal [12F7] to beta Catenin
Host species	Mouse
Tested applications	Suitable for: IHC-P, WB, Flow Cyt (Intra)
Species reactivity	Reacts with: Human Predicted to work with: Mouse, Rat, Pig 
Immunogen	Recombinant full length protein corresponding to Chicken beta Catenin.
Positive control	In Western Blot, ab22656 gave a positive signal in the following human whole cell lysates: HeLa; DU145; A549; A431. IHC-P - Human colon adenocarcinoma FFPE tissue sections
General notes	<p>This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com.</p> <p>The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets your needs before purchasing.</p> <p>If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be found below, along with publications, customer reviews and Q&As</p>

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.40 Preservative: 0.02% Sodium azide Constituent: PBS
Purity	Protein G purified
Clonality	Monoclonal

Clone number 12F7
 Isotype IgG1

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab22656 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
IHC-P	★★★★★ (2)	Use a concentration of 1 - 5 µg/ml. Perform heat mediated antigen retrieval via the microwave method before commencing with IHC staining protocol.
WB	★★★★☆ (2)	Use a concentration of 2 µg/ml. Detects a band of approximately 85 kDa (predicted molecular weight: 85 kDa). We recommend using 3% milk as the blocking agent for Western blot.
Flow Cyt (Intra)		Use 1µg for 10 ⁶ cells. ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

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.

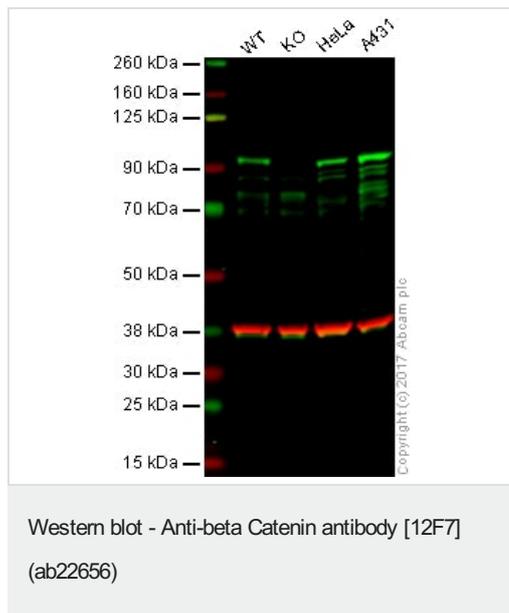
Ubiquitinated by the SCF(BTRC) E3 ligase complex when phosphorylated by GSK3B, leading to its degradation. Ubiquitinated by a E3 ubiquitin ligase complex containing UBE2D1, SIAH1, CACYBP/SIP, SKP1, APC and TBL1X, leading to its subsequent proteasomal degradation.

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



Lane 1: Wild type HAP1 whole cell lysate (20 µg)

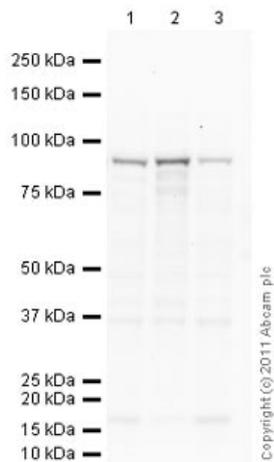
Lane 2: CTNNB1 knockout HAP1 whole cell lysate (20 µg)

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 - ab22656 observed at 95 kDa. Red - loading control, [ab181602](#), observed at 37 kDa.

ab22656 was shown to specifically react with CTNNB1 (beta-catenin) in wild-type HAP1 cells along with additional cross reactive bands. No band was observed when CTNNB1 knockout samples were used. Wild-type and CTNNB1 knockout samples were subjected to SDS-PAGE. Ab22656 and [ab181602](#) (Rabbit 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-Mouse IgG H&L (IRDye® 800CW) preabsorbed [ab216772](#) and Goat anti-Rabbit IgG H&L (IRDye® 680RD) preabsorbed [ab216777](#) secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-beta Catenin antibody [12F7] (ab22656)

All lanes : Anti-beta Catenin antibody [12F7] (ab22656) at 2 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell Lysate

Lane 2 : DU 145 (Human prostate carcinoma cell line) Whole Cell Lysate

Lane 3 : A549 (Human lung adenocarcinoma epithelial cell line) Whole Cell Lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Mouse IgG H&L (HRP) preadsorbed (**ab97040**) at 1/5000 dilution

Developed using the ECL technique.

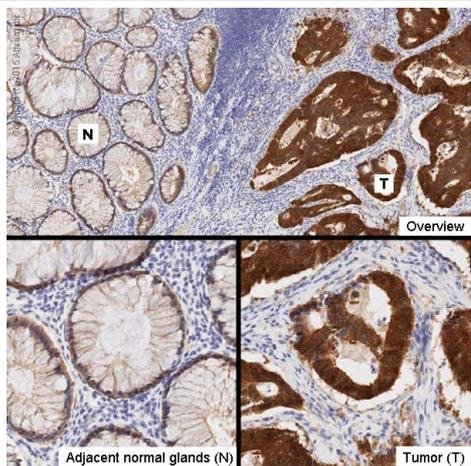
Performed under reducing conditions.

Predicted band size: 85 kDa

Observed band size: 85 kDa

Exposure time: 12 minutes

We recommend using 3% milk as the blocking agent for Western blot.

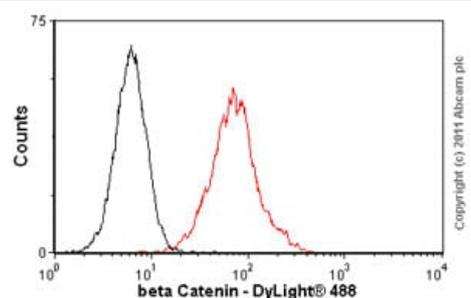


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Catenin antibody [12F7] (ab22656)

IHC image of ab22656 staining beta Catenin in human colon adenocarcinoma formalin-fixed paraffin-embedded tissue sections*, 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. The section was then incubated with ab22656, 1/500 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. High magnification of the tumor region - T (lower right panel) and adjacent normal crypts - N (lower left panel) are shown.

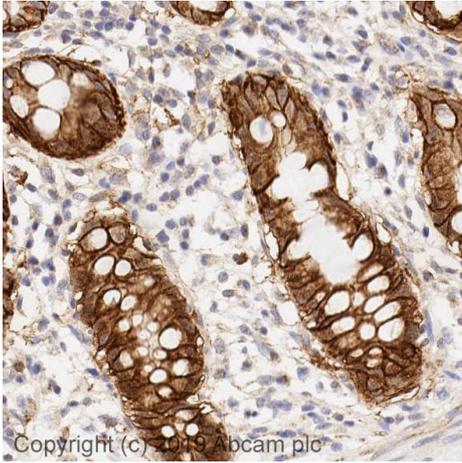
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

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



Flow Cytometry (Intracellular) - Anti-beta Catenin antibody [12F7] (ab22656)

Overlay histogram showing HeLa cells stained with ab22656 (red line). The cells were fixed with methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab22656, 1µg/1x10⁶ cells) for 30 min at 22°C. The secondary antibody used was DyLight[®] 488 goat anti-mouse IgG (H+L) ([ab96879](#)) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG1 [ICIGG1] ([ab91353](#), 2µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a decreased signal in HeLa cells fixed with 4% paraformaldehyde (10 min)/permeabilized in 0.1% PBS-Tween used under the same conditions.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Catenin antibody [12F7] (ab22656)

IHC image of beta Catenin antibody staining in a section of formalin-fixed paraffin-embedded normal human colon* performed on a Leica BOND™ system using the standard protocol. 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 ab22656, 1ug/ml, 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.

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 [12F7] (ab22656)

This image is courtesy of an anonymous Abreview

ab22656 staining rat bowel tissue section by IHC-P. The section was formaldehyde fixed and subjected to heat mediated antigen retrieval in citrate buffer (pH6) prior to blocking with 10% serum for 10 hours at 24°C. The primary antibody was diluted 1/500 in milk and incubated with the section for 12 hours at 4°C. A biotinylated rabbit anti-mouse was used as the second antibody.

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