abcam

Product datasheet

Anti-beta Catenin antibody [E247] - BSA and Azide free ab196204

KO VALIDATED Recombinant RabMAb

★★★★★ 1 Abreviews 33 References 25 Images

Overview

Product name	Anti-beta Catenin antibody [E247] - BSA and Azide free
Description	Rabbit monoclonal [E247] to beta Catenin - BSA and Azide free
Host species	Rabbit
Specificity	This antibody is not suitable for ICC testing in mouse and rat species. Our inhouse testing indicated that this antibody does not work in Raw264.7 cell line in western blot. We have an alternative antibody **ab68183** detecting weak band in lower expressor Raw264.7.
Tested applications	**Suitable for:** IHC-P, IP, WB, ChIP, ICC/IF **Unsuitable for:** Flow Cyt
Species reactivity	**Reacts with:** Mouse, Rat, Human **Predicted to work with:** Sheep, Hamster, Cow, Macaque monkey, African green monkey
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: A431, HeLa, wild-type HAP1, HCT 116 and Wild-type MCF7 cell lysates. ICC/IF: A431 and wild-type HAP1 cells. SW480 and SK-N-SH cells. IHC-P: Human lung adenocarcinoma, kidney adenocarcinoma, colon adenocarcinoma, cervical carcinoma, breast carcinoma and papillary carcinoma of thyroid gland tissue, Rat liver and pancreas, Mouse liver and pancreas; IP: A431 whole cell lysate and mouse brain lysate.
General notes	ab196204 is the carrier-free version of **ab32572**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the

need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

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. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	E247
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab196204 in the following tested applications.

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

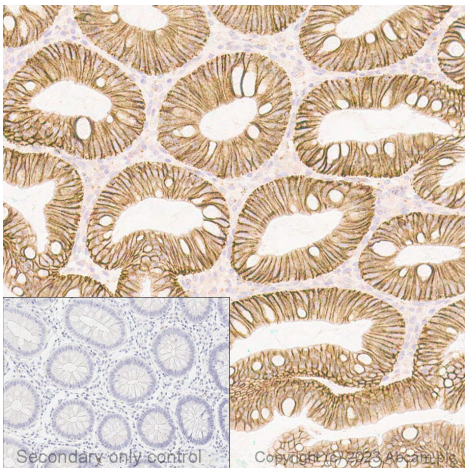
Application	Abreviews	Notes
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 92 kDa (predicted molecular weight: 85 kDa).
ChIP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.

Application notes Is unsuitable for Flow Cyt.

Target

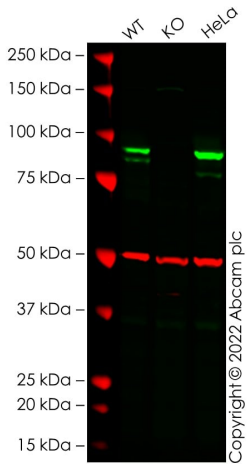
Function	Key dowstream component of the canonical Wnt signaling pathway. In the absence of Wnt, forms a complex with AXIN1, AXIN2, APC, CSNK1A1 and GSK3B that promotes phosphorylation on N-terminal Ser and Thr residues and ubiquitination of CTNNB1 via BTRC and its subsequent degradation by the proteasome. 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



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

Immunohistochemical analysis of formalin fixed paraffin embedded human tonsil labelling beta catenin with ab196204 at a concentration of 1µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32 min with ULTRA cell conditioning solution (CC1 pH 8.5). ab196204 anti beta catenin antibody was incubated at 37°C for 16min. Sections were counterstained is with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control



Western blot - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

All lanes : Anti-beta Catenin antibody [E247] - ChIP Grade (**ab32572**) at 1/5000 dilution

Lane 1 : Wild-type MCF7 cell lysate
Lane 2 : CTNNB1 knockout MCF7 cell lysate
Lane 3 : HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution

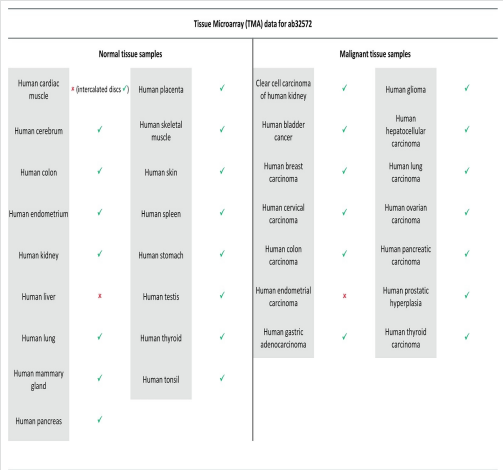
Performed under reducing conditions.

Predicted band size: 85 kDa
Observed band size: 85/90 kDa

False colour image of Western blot: Anti-beta Catenin antibody [E247] - ChIP Grade staining at 1/5000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab32572** was shown to bind specifically to beta Catenin. A band was observed at

85/90 kDa in wild-type MCF7 cell lysates with no signal observed at this size in CTNNB1 knockout cell line **ab286762**. To generate this image, wild-type and CTNNB1 knockout MCF7 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 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32572**).



Tissue Microarray (TMA) data for ab32572

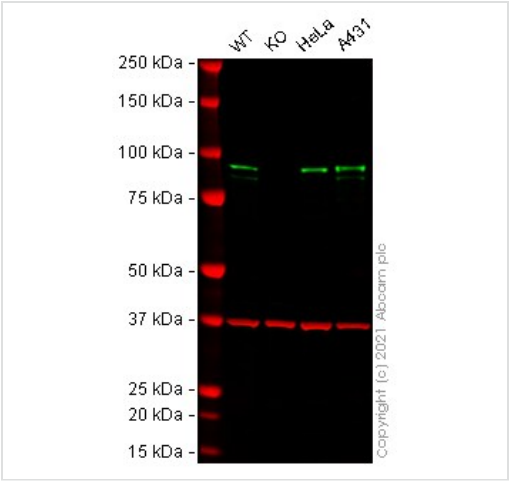
Normal tissue samples				Malignant tissue samples			
Human cardiac muscle	x (intercalated discs ✓)	Human placenta	✓	Clear cell carcinoma of human kidney	✓	Human glioma	✓
Human cerebrum	✓	Human skeletal muscle	✓	Human bladder cancer	✓	Human hepatocellular carcinoma	✓
Human colon	✓	Human skin	✓	Human breast carcinoma	✓	Human lung carcinoma	✓
Human endometrium	✓	Human spleen	✓	Human cervical carcinoma	✓	Human ovarian carcinoma	✓
Human kidney	✓	Human stomach	✓	Human colon carcinoma	✓	Human pancreatic carcinoma	✓
Human liver	x	Human testis	✓	Human endometrial carcinoma	x	Human prostatic hyperplasia	✓
Human lung	✓	Human thyroid	✓	Human gastric adenocarcinoma	✓	Human thyroid carcinoma	✓
Human mammary gland	✓	Human tonsil	✓				
Human pancreas	✓						

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32572**).

Tissue Microarrays stained for Anti-beta Catenin antibody [E247] - ChIP Grade using **ab32572** in immunohistochemical analysis. This table provides a detailed overview of positive (tick mark) and negaive (cross mark) staining per sample type tested. The section was incubated with **ab32572** for 30 mins at room temperature followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). The immunostaining was performed on a Leica Biosystems BOND® RX instrument.

Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) for 20 mins.



Western blot - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

All lanes : Anti-beta Catenin antibody [E247] - ChIP Grade (**ab32572**) at 1/5000 dilution

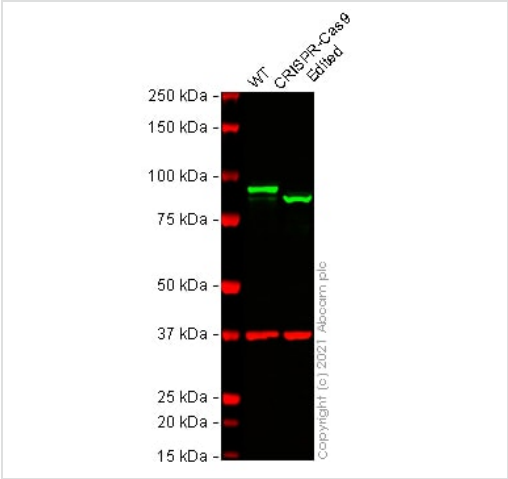
Lane 1 : Wild-type HepG2 cell lysate
Lane 2 : CTNNB1 knockout HepG2 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : A431 cell lysateA431 cell lysateA431 cell lysateA431 cell lysateA431 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

False colour image of Western blot: Anti-beta Catenin antibody [E247] - ChIP Grade staining at 1/5000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab32572** 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 (**ab277911**). 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 [E247] - BSA and Azide free (ab196204)

All lanes : Anti-beta Catenin antibody [E247] - ChIP Grade (**ab32572**) at 1/5000 dilution

Lane 1 : Wild-type HCT 116 cell lysate
Lane 2 : CTNNB1 CRISPR-Cas9 edited HCT 116 cell lysate

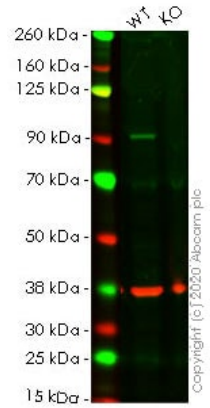
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 85 kDa
Observed band size: 95 kDa

False colour image of Western blot: Anti-beta Catenin antibody [E247] - ChIP Grade staining at 1/5000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab32572** 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 5 % 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 [E247] - BSA and Azide free (ab196204)

All lanes : Anti-beta Catenin antibody [E247] - ChIP Grade (**ab32572**) at 1/500 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : CTNNB1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

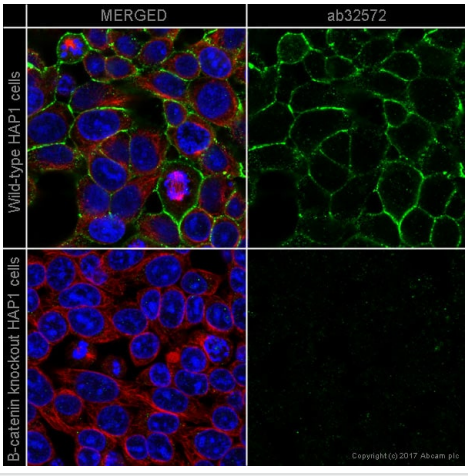
Performed under reducing conditions.

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

This data was developed using the same antibody clone in a different buffer formulation (**ab32572**).

Lanes 1- 2: Merged signal (red and green). Green - **ab32572** observed at 86 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab32572 was shown to react with beta Catenin in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line **ab255352** (knockout cell lysate **ab263756**) was used. Wild-type HeLa and CTNNB1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. **ab32572** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 500 Dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

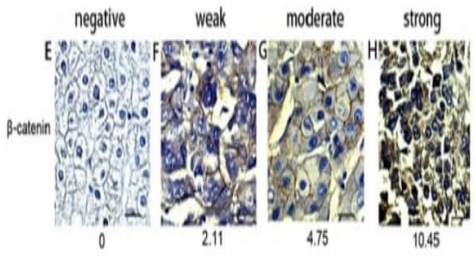


Immunocytochemistry/ Immunofluorescence - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

ab32572 staining in CTNNB1 (beta Catenin) wild-type HAP1 cells (top panel) and in 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 **ab32572** 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 **Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081) secondary antibody** at 2 µg/ml (shown in green). Nuclear DNA was labeled in blue with DAPI.

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32572**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

Image from Jin et al PLoS One. 2015 Aug 7;10(8):e0133770. doi: 10.1371/journal.pone.0133770. eCollection 2015. Fig 2.

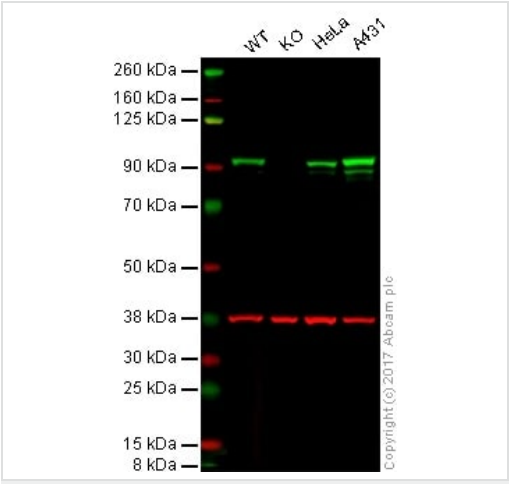
Different expression level of beta Catenin in Human HCTs (hepatocellular carcinoma tissues) and PLTs (para-cancerous liver tissues).

The HCTs, PLTs were paraffin-embedded and cut into sections with 5 µm-thickness for hematoxylin-eosin and immunohistochemistry (IHC) analysis. **ab32572** was used at a dilution of 1:400. The second antibody was a biotinylated IgG to incubate 40 minutes at 37°C. Finally, the tissue slices were visualized by the 3, 3-diaminobenzidine solution and counterstained with hematoxylin. Substitution of the primary antibody with phosphate-buffered saline was served as a control for IHC.

The beta Catenin with negative, weak, moderate and strong staining activity was respectively detected in HCTs (E-H) and PLTs (M-P). Section E shown above, for full image please see original paper.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and

sodium azide (**ab32572**).



Western blot - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

All lanes : Anti-beta Catenin antibody [E247] - ChIP Grade (**ab32572**) at 1/5000 dilution

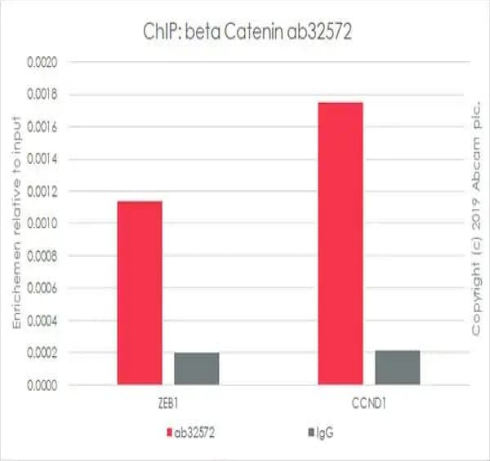
Lane 1 : Wild-type HAP1 whole cell lysate
Lane 2 : CTNNB1 (ß-catenin) knockout HAP1 whole cell lysate
Lane 3 : HeLa whole cell lysate
Lane 4 : A431 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 85 kDa

Lanes 1 - 4: Merged signal (red and green). Green - **ab32572** observed at 90 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab32572 was shown to specifically react with CTNNB1 (β-catenin) in wild type HAP1 cells. No band was observed when CTNNB1 (β-catenin) knockout samples were used. Wild-type and CTNNB1 (β-catenin) knockout samples were subjected to SDS-PAGE. **ab32572** and **ab8245** (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at a 1/5000 dilution and 1/10000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



ChIP - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

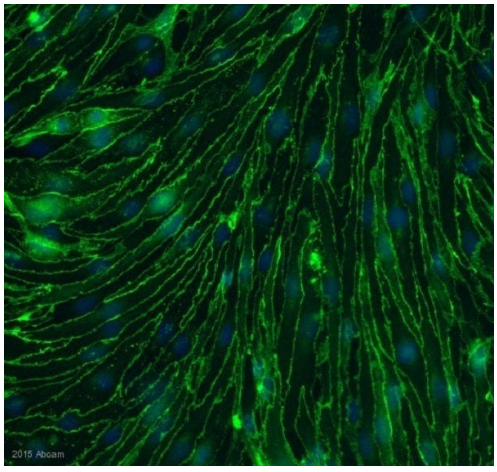
This data was developed using the same antibody in a different buffer formulation (**ab32572**).

Chromatin was prepared from HCT 116 cells according to the Abcam Dual-X-ChIP protocol*. Cells were fixed with 1.5 mM EGS for 30 mins and then formaldehyde for 10 min.

The ChIP was performed with 25 µg of chromatin, 5 µg of **ab32572** (red), or 5 µg of rabbit normal IgG **ab172730** (gray) and 20 µl of Protein A/G sepharose beads. The immunoprecipitated DNA was quantified by real time PCR (Taqman approach for active and inactive loci, Sybr green approach for heterochromatic loci)

Primers and probes are from paper PMID: 28625518

*http://www.abcam.com/resources?keywords=X%20ChIP%20protocol



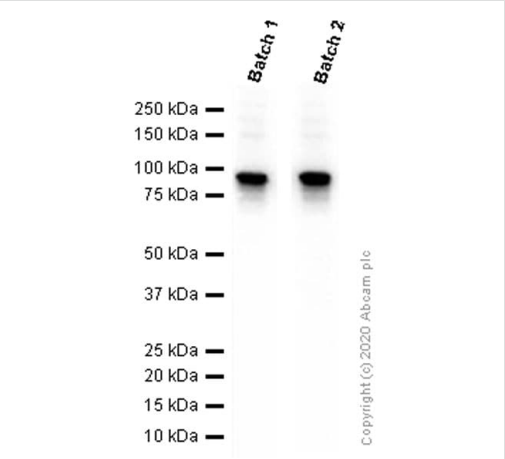
Immunocytochemistry/ Immunofluorescence - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

This image is courtesy of an anonymous Abreview.

ab32572 staining beta Catenin in the bEnd.5 murine cell line by ICC/IF (Immunocytochemistry/immunofluorescence).

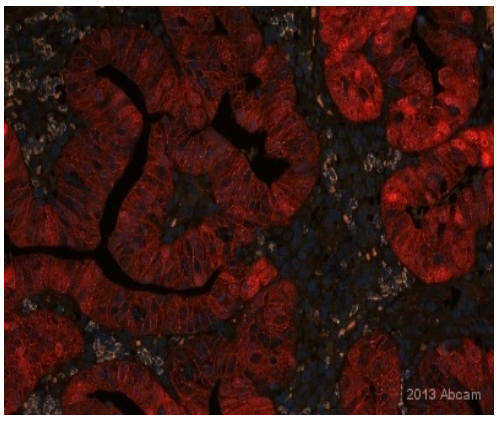
Cells were fixed with paraformaldehyde, permeabilized with 0.1% Triton in PBS and blocked with 10% serum for 30 minutes at 22°C. Samples were incubated with primary antibody (1/300) for 16 hours at 4°C. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG polyclonal (1/500) was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32572**).



Western blot - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

This data was developed using **ab32572**, the same antibody clone in a different buffer formulation. Different batches of **ab32572** were tested on A431 (Human epidermoid carcinoma epithelial cell) lysate at 2.0 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 92 kDa.



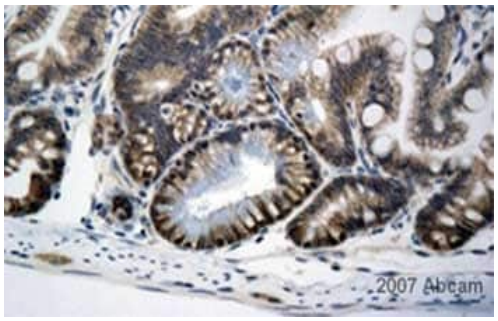
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

This image is courtesy of an anonymous Abreview.

ab32572 staining beta Catenin in dog colon tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections).

Tissue was fixed with formaldehyde and blocked with 10% serum for 30 minutes at 25°C; antigen retrieval was by heat mediation in 10 mM citrate buffer, pH6. Samples were incubated with primary antibody (1/250 in PBS with 1x casein) for 90 minutes at 25°C. A biotin-conjugated Goat anti-rabbit IgG polyclonal (1/200) was used as the secondary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32572**).



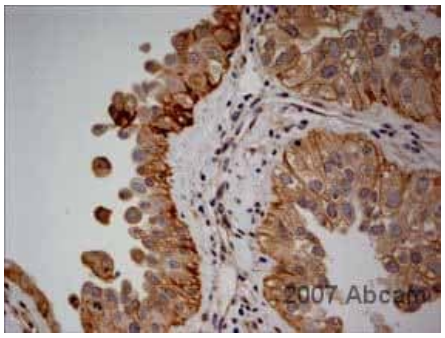
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

This image is courtesy of an anonymous Abreview.

ab32572 at 1/200 staining mouse small intestine tissue sections by IHC-P.

The tissue was formaldehyde fixed and a heat mediated antigen retrieval step was performed before incubation with the primary antibody. An HRP conjugated goat anti-rabbit antibody was used as the secondary.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32572**).



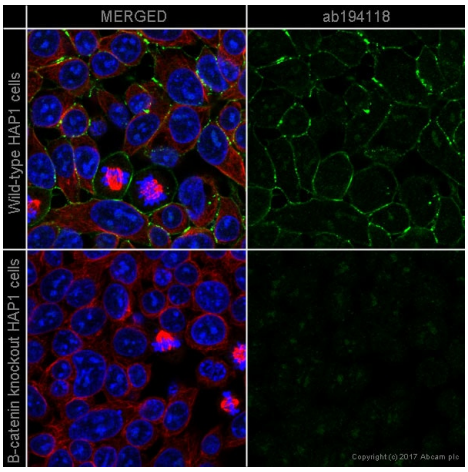
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

This image is courtesy of an anonymous Abreview.

ab32572 staining human renal carcinoma tissue sections by IHC-P.

Sections were formaldehyde fixed and subjected to heat mediated antigen retrieval in citrate buffer (pH 6) prior to blocking with 1% milk for 45 minutes at 22°C. The primary antibody was diluted 1/200 and incubated with the sample for 1 hour at 22°C. An HRP conjugated goat anti-rabbit antibody, diluted 1/400, was used as the secondary.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32572**).

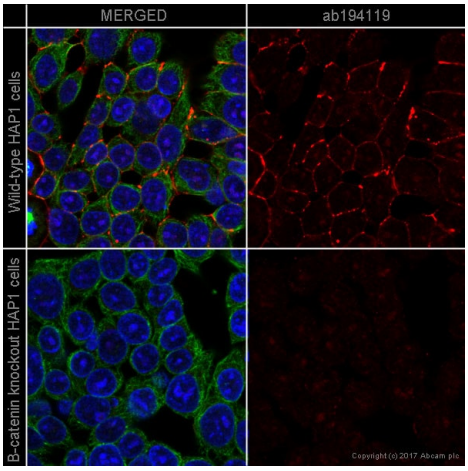


Immunocytochemistry/ Immunofluorescence - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

Clone E247 (ab196204) has been successfully conjugated by Abcam. This image was generated using Anti-beta Catenin antibody [E247] (Alexa Fluor® 488). Please refer to **ab194118** for protocol details.

ab194118 staining β-catenin in wild-type HAP1 cells (top panel) and 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 **ab194118** at 1/500 dilution (shown in green) and **ab195889** at 1/250 dilution (shown in pseudo colour red) overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

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

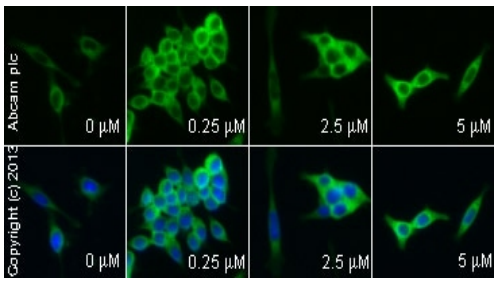


Immunocytochemistry/ Immunofluorescence - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

Clone E247 (ab196204) has been successfully conjugated by Abcam. This image was generated using Anti-beta Catenin antibody [E247] (Alexa Fluor® 647). Please refer to **ab194119** for protocol details.

ab194119 staining β-catenin in wild-type HAP1 cells (top panel) and 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 **ab194119** at 1/500 dilution (shown in red) and **ab195887** at 1/250 dilution (shown in green) overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

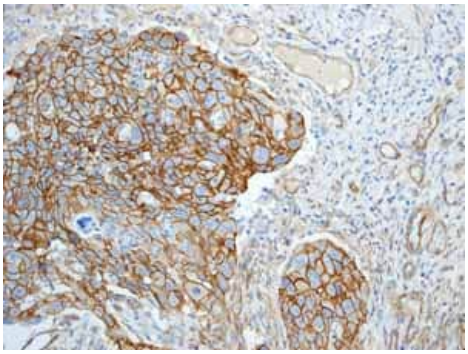
Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

ab32572 staining beta Catenin in SW480 (Human colorectal adenocarcinoma cell line) cells treated with BIO (**ab120891**), by ICC/IF. Increase of beta Catenin expression correlates with increased concentration of BIO, as described in literature.
The cells were incubated at 37°C for 48h in media containing different concentrations of **ab120891** (BIO) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with **ab32572** (1/200) dilution was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A **Goat Anti-Rabbit IgG H&L (DyLight® 488) preadsorbed (ab96899) secondary antibody** at 1/250 dilution was used as the secondary antibody. Nuclei were counterstained with DAPI and are shown in blue.

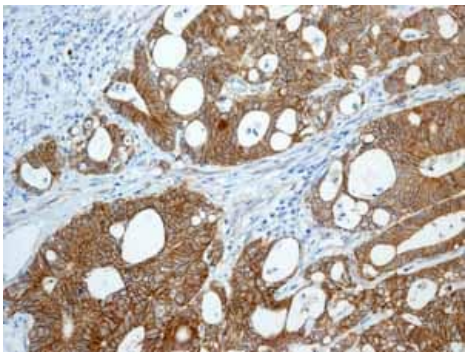
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32572**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

ab32572 showing positive staining in human cervical carcinoma tissue.

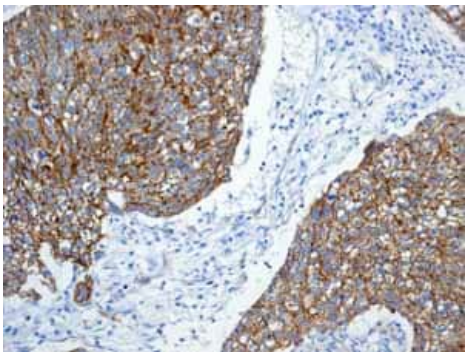
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32572**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

ab32572 showing positive staining in human breast carcinoma tissue.

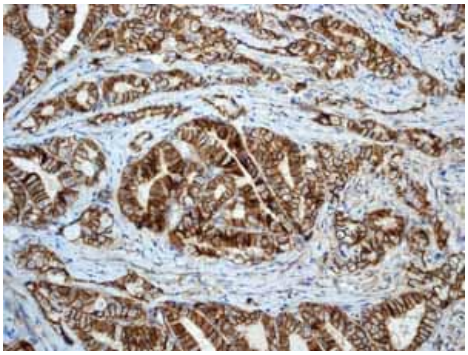
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32572**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

ab32572 showing positive staining in human lung adenocarcinoma tissue.

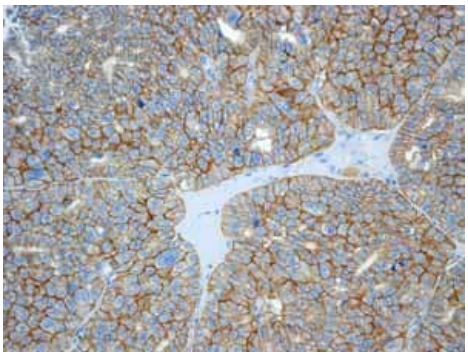
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32572**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

ab32572 showing positive staining in human papillary carcinoma of thyroid gland tissue.

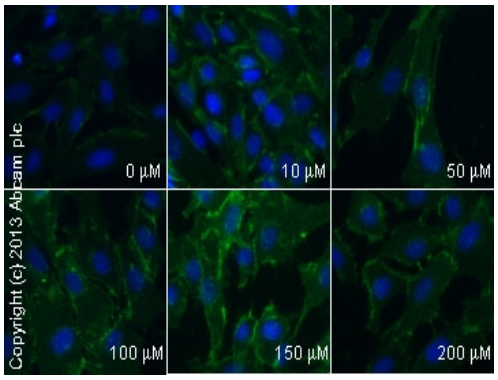
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32572**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

ab32572 showing positive staining in human kidney carcinoma tissue.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32572**).



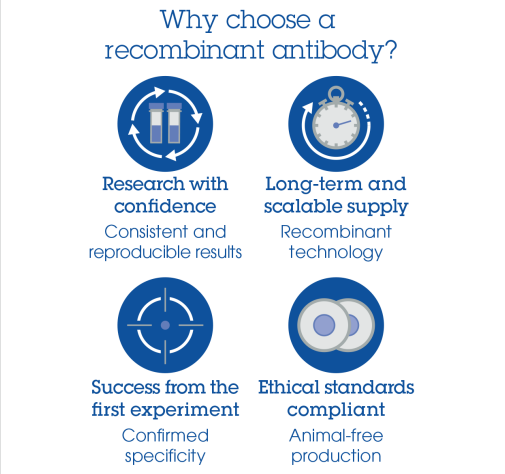
Immunocytochemistry/ Immunofluorescence - Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

ab32572 staining beta Catenin in SK-N-SH (Human neuroblastoma cell line) cells treated with olanzapine (**ab120736**), by ICC/IF.

Increase in expression of beta Catenin correlates with increased concentration of olanzapine, as described in literature.
The cells were incubated at 37°C for 24h in media containing different concentrations of **ab120736** (olanzapine) in DMSO, fixed with 4% formaldehyde for 10 minutes at room temperature and blocked with PBS containing 10% goat serum, 0.3 M glycine, 1% BSA and 0.1% tween for 2h at room temperature. Staining of the treated cells with **ab32572** (1/200 dilution) was performed overnight at 4°C in PBS containing 1% BSA and 0.1% tween. A **Goat Anti-Rabbit IgG H&L (DyLight® 488) preadsorbed (ab96899) secondary antibody** at 1/250 dilution was used as the secondary

antibody. Nuclei were counterstained with DAPI and are shown in blue.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab32572**).



Anti-beta Catenin antibody [E247] - BSA and Azide free (ab196204)

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