abcam

Product datasheet

Human CTNNB1 (beta Catenin) knockout Hep G2 cell line ab277911

3 Images

Overview

Product name	Human CTNNB1 (beta Catenin) knockout Hep G2 cell line
Parental Cell Line	HepG2
Organism	Human
Passage number	<20
Knockout validation	Western Blot (WB)
Tested applications	**Suitable for:** WB
Biosafety level	1
General notes	**Recommended control:** Human wild-type HepG2 cell line (**ab275467**). Please note a wild-type cell line is not automatically included with a knockout cell line order, if required please add recommended wild-type cell line at no additional cost using the code WILDTYPE-TMTK1. **Cryopreservation cell medium:** Cell Freezing Medium-DMSO Serum free media, contains 8.7% DMSO in MEM supplemented with methyl cellulose. **Culture medium:** MEM + 10% FBS **Initial handling guidelines:** Upon arrival, the vial should be stored in liquid nitrogen vapor phase and not at -80°C. Storage at -80°C may result in loss of viability. 1. Thaw the vial in 37°C water bath for approximately 1-2 minutes. 2. Transfer the cell suspension (0.8 mL) to a 15 mL/50 mL conical sterile polypropylene centrifuge tube containing 8.4 mL pre-warmed culture medium, wash vial with an additional 0.8 mL culture medium (total volume 10 mL) to collect remaining cells, and centrifuge at 201 x g (rcf) for 5 minutes at room temperature. 10 mL represents minimum recommended dilution. 20 mL represents maximum recommended dilution. 3. Resuspend the cell pellet in 5 mL pre-warmed culture medium and count using a haemocytometer or alternative cell counting method. Based on cell count, seed cells in an appropriate cell culture flask at a density of $2x10^4$ cells/cm^2. Seeding density is given as a guide only and should be scaled to align with individual lab schedules. 4. Incubate the culture at 37°C incubator with 5% CO_2. Cultures should be monitored daily. **Subculture guidelines:** All seeding densities should be based on cell counts gained by established methods. A guide seeding density of $2x10^4$ cells/cm^2 is recommended. A partial media change 24 hours prior to subculture may be helpful to encourage growth, if

required.
Cells should be passaged when they have achieved 80-90% confluence.
This product is subject to limited use licenses from The Broad Institute and ERS Genomics Limited, and is developed with patented technology. For full details of the limited use licenses and relevant patents please refer to our **limited use license** and **patent pages**.

We will provide viable cells that proliferate on revival.

Properties

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Liver
Cell type	epithelial
Disease	Hepatocellular Carcinoma
Gender	Male
Mycoplasma free	Yes
Storage instructions	Shipped on Dry Ice. Store in liquid nitrogen.
Storage buffer	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

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.

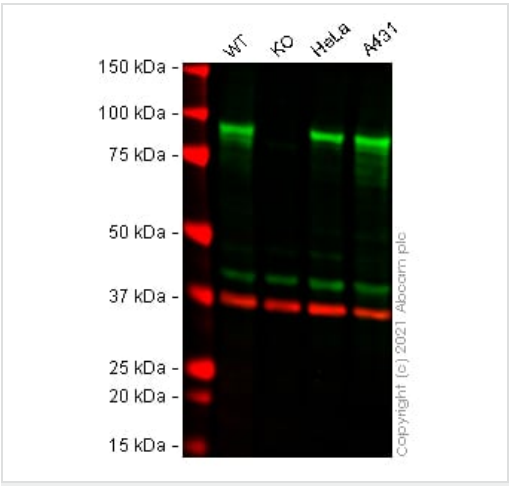
Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab277911 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 at an assay dependent concentration.

Images



Western blot - Human CTNNB1 (beta Catenin) knockout Hep G2 cell line (ab277911)

All lanes : Anti-beta Catenin non-phospho(active) S37/T41 antibody [EPR23969-131] (**ab246504**) at 1/1000 dilution

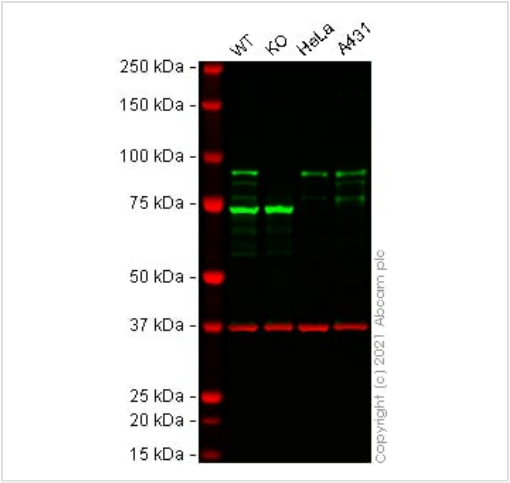
Lane 1 : Wild-type HepG2 cell lysate
Lane 2 : CTNNB1 knockout HepG2 cell lysate
Lane 3 : HeLa cell lysate
Lane 4 : A431 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Observed band size: 90 kDa

False colour image of Western blot: Anti-beta Catenin non-phospho(active) S37/T41 antibody [EPR23969-131] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab246504** was shown to bind specifically to beta Catenin non-phospho(active) S37/T41. A band was observed at 90 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 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 - Human CTNNB1 (beta Catenin) knockout Hep G2 cell line (ab277911)

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

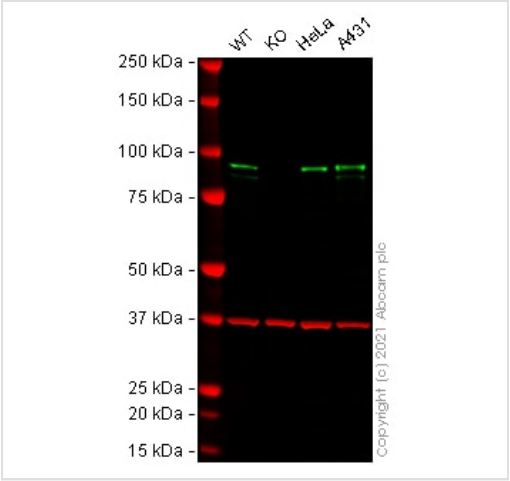
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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 - Human CTNNB1 (beta Catenin) knockout Hep G2 cell line (ab277911)

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 lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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.

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