abcam

Product datasheet

Human CTNNB1 (beta Catenin) knockout HCT116 cell lysate ab275247

画像数 6

製品の概要

製品名	Human CTNNB1 (beta Catenin) knockout HCT116 cell lysate
製品の概要	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HCT116
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 14 bp deletion and 7 bp insertion in exon 3
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
Reconstitution notes	To use as WB control, resuspend the lyophilizate in 50 µL of LDS* Sample Buffer to have a final concentration of 2 mg/ml. For reducing conditions, we recommend a final concentration of 0.1 M DTT. **Usage of SDS sample buffer is not recommended with these lyophilized lysates.*
特記事項	**Lysate preparation:** Our lysates are made using RIPA buffer to which we add a protease inhibitor cocktail and phosphatase inhibitor cocktail (ratio: 300:100:10). *This means that the protein of interest is denatured.* If you require a native form of the protein please use the live cell version - found **here**. Please refer to our lysis protocol for further details on how our lysates are prepared. **User storage instructions:** Lyophilizate may be stored at 4°C. After reconstitution, store at -20°C for short-term storage or -80°C for long-term storage. Access thousands of knockout cell lysates, generated from commonly used cancer cell lines. **See here for more information on knockout cell lysates.** Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances. It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses. This product is subject to limited use licenses from The Broad Institute, ERS Genomics Limited and Sigma-Aldrich Co. LLC, and is developed with patented technology. For full details of the licenses and patents please refer to our **limited use license** and **patent pages**.

アプリケーション **適用あり:** WB

製品の特性

保存方法 Store at -80°C. Please refer to protocols.

内容	1 kit
ab277291 - Human CTNNB1 knockout HCT116 cell lysate	1 x 100µg
ab277289 - Human wild-type HCT116 cell lysate	1 x 100µg

Cell type epithelial

Disease Carcinoma

Gender Male

ターゲット情報

機能 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.

組織特異性 Expressed in several hair follicle cell types: basal and peripheral matrix cells, and cells of the outer and inner root sheaths. Expressed in colon.

関連疾患 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.

配列類似性 Belongs to the beta-catenin family.
Contains 12 ARM repeats.

翻訳後修飾	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.
細胞内局在	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.

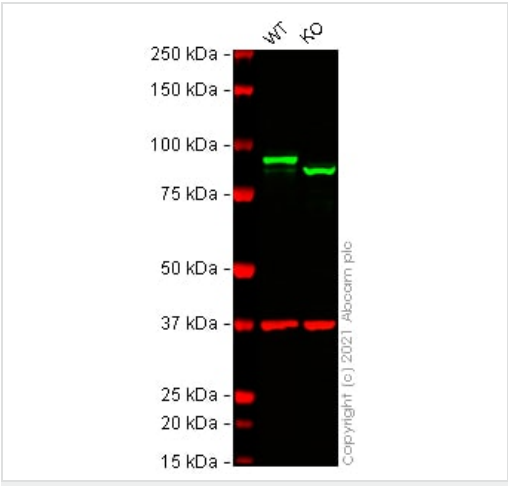
アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab275247の使用に適用されます

アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration. Predicted molecular weight: 85 kDa.

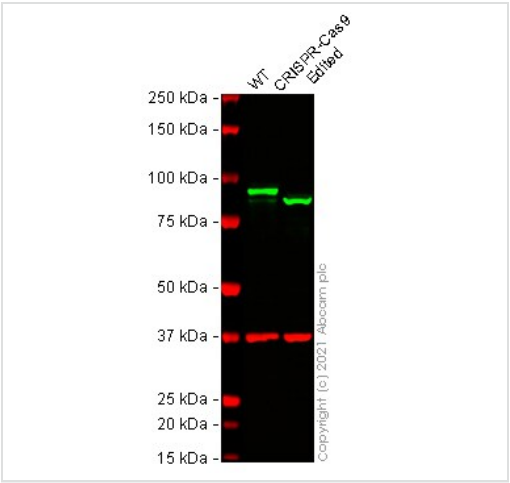
画像



Western blot - Human CTNNB1 (beta Catenin) knockout HCT116 cell lysate (ab275247)

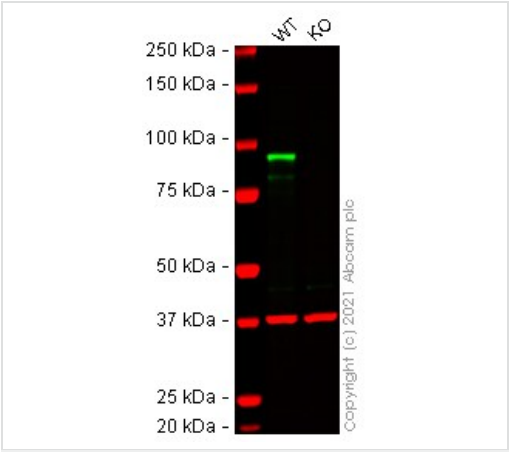
Lane 1: Wild-type HCT 116 cell lysate 20 µg
Lane 2: CTNNB1 knockout HCT 116 cell lysate 20 µg
False colour image of Western blot: Anti-beta Catenin antibody [E247] - ChIP Grade staining at 1/5000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining at 1/20000 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 knockout cell line **ab273712** (knockout cell lysate ab275247). The band observed in the knockout 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 knockout 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 - Human CTNNB1 (beta Catenin) knockout HCT116 cell lysate (ab275247)

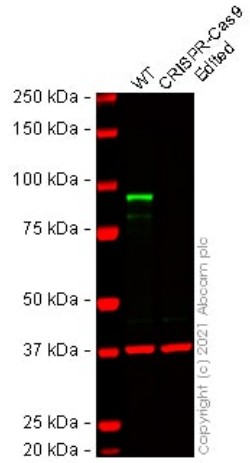
Lane 1: Wild-type HCT 116 cell lysate 20 µg
Lane 2: CTNNB1 CRISPR-Cas9 edited HCT 116 cell lysate 20 µg
False colour image of Western blot: Anti-beta Catenin antibody [E247] - ChIP Grade staining at 1/5000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (**ab8245**) loading control staining 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 - Human CTNNB1 (beta Catenin) knockout HCT116 cell lysate (ab275247)

Lane 1: Wild-type HCT 116 cell lysate 20 µg
Lane 2: CTNNB1 knockout HCT 116 cell lysate 20 µg
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 knockout cell line **ab273712** (knockout cell lysate ab275247). The band observed in the knockout 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 knockout 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.

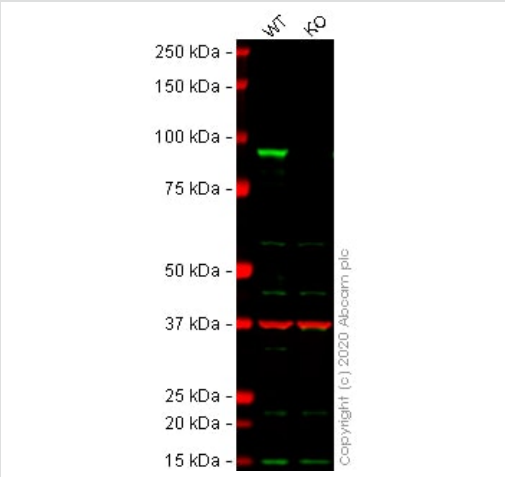


Western blot - Human CTNNB1 (beta Catenin) knockout HCT116 cell lysate (ab275247)

Lane 1: Wild-type HCT 116 cell lysate 20 µg

Lane 2: CTNNB1 CRISPR-Cas9 edited HCT 116 cell lysate 20 µg

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.



Western blot - Human CTNNB1 (beta Catenin) knockout HCT116 cell lysate (ab275247)

Lane 1: Wild-type HCT116 cell lysate 20 ug

Lane 2: CTNNB1 knockout HCT116 cell lysate 20 ug

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 wild-type HCT 116 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 fluoroscent 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 ug/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.

Sanger Sequencing - Human CTNNB1 (beta Catenin) knockout HCT116 cell lysate (ab275247)

Allele-1: 7 bp insertion and 14 bp deletion in exon 3.

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