

Human CTNNB1 (beta Catenin) knockout HeLa cell lysate ab263756

画像数 3

製品の概要

製品名	Human CTNNB1 (beta Catenin) knockout HeLa cell lysate
製品の概要	Knockout cell lysate achieved by CRISPR/Cas9.
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp deletion 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. <i>*Usage of SDS sample buffer is not recommended with these lyophilized lysates.</i>

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.](#)

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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
ab255460 - Human CTNNB1 knockout HeLa cell lysate	1 x 100µg
ab255552 - Human wild-type HeLa cell lysate	1 x 100µg

Cell type epithelial
Disease Adenocarcinoma
Gender Female
STR Analysis Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 WWA: 16, 18 TH01: 7 TPOX: 8, 12 CSF1PO: 9, 10

ターゲット情報

機能 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.

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 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.

配列類似性 Belongs to the beta-catenin family.

翻訳後修飾

Contains 12 ARM repeats.

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

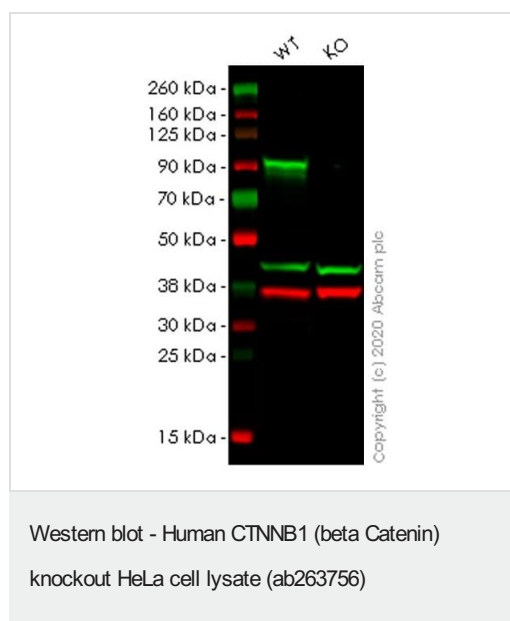
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アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB		Use at an assay dependent concentration.

画像



Blocking and diluting buffer and concentration: Intercept® (TBS)

Blocking Buffer diluted with an equal volume of 0.1% TBST

The molecular weight observed is consistent with what has been described in the literature (PMID: 16288032).

Lane 1: Wild-type HeLa cell lysate (20µg)

Lane 2: CTNNB1 knockout HeLa cell lysate (20µg)

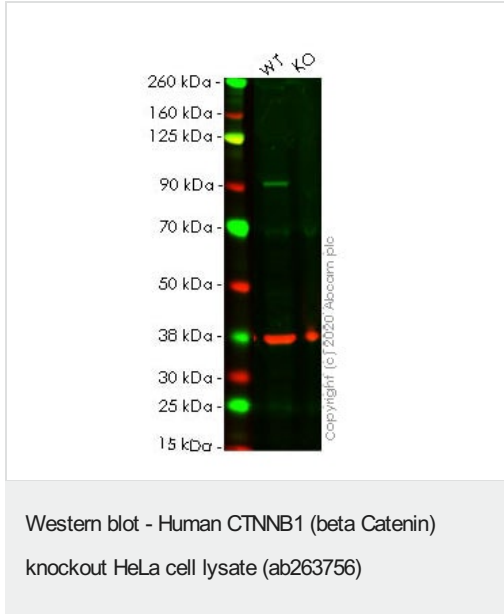
Lanes 1-2: Merged signal (red and green). Green - **ab246504** observed at 92 kDa. Red - loading control **ab8245** (Mouse monoclonal [6C5] to GAPDH) observed at 36 kDa.

ab246504 Anti-beta Catenin (non-phospho(active) S37/T41) antibody [EPR23969-131] was shown to specifically react with beta Catenin in wild-type HeLa cells. Loss of signal was observed when knockout cell line **ab255352** (knockout cell lysate ab263756) was used.

Wild-type and beta Catenin knockout samples were subjected to SDS-PAGE. **ab246504** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 dilution and 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 10000 dilution for 1 hour at room temperature before imaging.

An extra band around 45 kDa was observed.

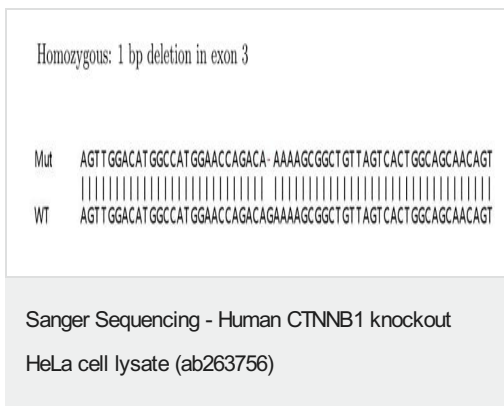


Lane 1: Wild-type HeLa cell lysate (20µg)

Lane 2: CTNNB1 knockout HeLa cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - **ab32572** observed at 86 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab32572 Recombinant Anti-beta Catenin antibody [E247] - ChIP Grade was shown to specifically react with CTNNB1 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 and CTNNB1 knockout samples 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**) were incubated overnight at 4 °C at 1 in 500 dilution and 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.



Homozygous: 1 bp deletion in exon 3

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