abcam

Product datasheet

Human NOTCH1 knockout HeLa cell line ab261762

3 图像

概述

产品名称	人NOTCH1 knockout HeLa cell line
Parental Cell Line	HeLa
Organism	Human
Mutation description	Knockout achieved by using CRISPR/Cas9, Homozygous: 1 bp insertion in exon 5
Passage number	<20
Knockout validation	Sanger Sequencing, Western Blot (WB)
经测试应用	**适用于:** WB
Biosafety level	2
常规说明	**Recommended control:** Human wild-type HeLa cell line (**ab255448**). 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:** DMEM (High Glucose) + 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.
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We will provide viable cells that proliferate on revival.

性能

Number of cells	1 x 10^6 cells/vial, 1 mL
Adherent /Suspension	Adherent
Tissue	Cervix
Cell type	epithelial
Disease	Adenocarcinoma
Gender	Female
STR Analysis	Amelogenin X D5S818: 11, 12 D13S317: 12, 13.3 D7S820: 8, 12 D16S539: 9, 10 vWA: 16, 18 TH01: 7 TPOX: 8, 12 CSF1PO: 9, 10
Antibiotic resistance	Puromycin 1.00µg/ml
Mycoplasma free	Yes
存放说明	Shipped on Dry Ice. Store in liquid nitrogen.
存储溶液	Constituents: 8.7% Dimethylsulfoxide, 2% Cellulose, methyl ether

靶标

功能	Functions as a receptor for membrane-bound ligands Jagged1, Jagged2 and Delta1 to regulate cell-fate determination. Upon ligand activation through the released notch intracellular domain (NICD) it forms a transcriptional activator complex with RBPJ/RBPSUH and activates genes of the enhancer of split locus. Affects the implementation of differentiation, proliferation and apoptotic programs. May be important for normal lymphocyte function. In altered form, may contribute to transformation or progression in some T-cell neoplasms. Involved in the maturation of both CD4+ and CD8+ cells in the thymus. May be important for follicular differentiation and possibly cell fate selection within the follicle. During cerebellar development, may function as a receptor for neuronal DNER and may be involved in the differentiation of Bergmann glia.
组织特异性	In fetal tissues most abundant in spleen, brain stem and lung. Also present in most adult tissues where it is found mainly in lymphoid tissues.
疾病相关	Defects in NOTCH1 are a cause of bicuspid aortic valve (BAV) [MIM:109730]. A common defect in the aortic valve in which two rather than three leaflets are present. It is often associated with aortic valve calcification and insufficiency. In extreme cases, the blood flow may be so restricted that the left ventricle fails to grow, resulting in hypoplastic left heart syndrome.
序列相似性	Belongs to the NOTCH family. Contains 5 ANK repeats. Contains 36 EGF-like domains. Contains 3 LNR (Lin/Notch) repeats.
翻译后修饰	Synthesized in the endoplasmic reticulum as an inactive form which is proteolytically cleaved by a furin-like convertase in the trans-Golgi network before it reaches the plasma membrane to yield an

active, ligand-accessible form. Cleavage results in a C-terminal fragment N(TM) and a N-terminal fragment N(EC). Following ligand binding, it is cleaved by TNF-alpha converting enzyme (TACE) to yield a membrane-associated intermediate fragment called notch extracellular truncation (NEXT). This fragment is then cleaved by presenilin dependent gamma-secretase to release a notch-derived peptide containing the intracellular domain (NICD) from the membrane. Phosphorylated.
O-glycosylated on the EGF-like domains. Contains both O-linked fucose and O-linked glucose.
Ubiquitinated; undergoes 'Lys-29'-linked polyubiquitination catalyzed by ITCH.

细胞定位 Cell membrane and Nucleus. Following proteolytical processing NICD is translocated to the nucleus.

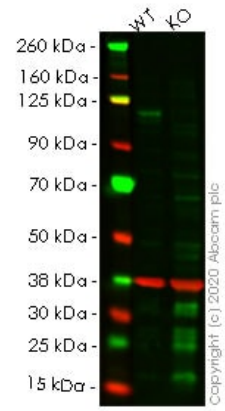
应用

The Abpromise guarantee **Abpromise™承诺保证使用**ab261762**于以下的经测试应用**

"应用说明"**部分 下显示的仅为推荐的起始稀释度**;实际**最佳的稀释度/浓度应由使用者检定。**

应用	Ab评论	说明
WB		Use at an assay dependent concentration. Predicted molecular weight: 272 kDa.

图片



Western blot - Human NOTCH1 knockout HeLa cell line (ab261762)

All lanes : Anti-Notch1 antibody (**ab65297**) at 1 µg/ml

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : NOTCH1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

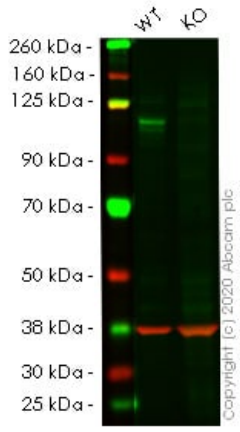
Performed under reducing conditions.

Predicted band size: 272 kDa
Observed band size: 110 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab65297** observed at 110 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab65297 was shown to react with Notch1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab261762 (knockout cell lysate **ab257006**) was used. Wild-type HeLa and NOTCH1 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. **ab65297** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at 1 µg/ml 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.



Western blot - Human NOTCH1 knockout HeLa cell line (ab261762)

All lanes : Anti-Notch1 antibody [EP1238Y] (**ab52627**) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : NOTCH1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 272 kDa
Observed band size: 110 kDa

Lanes 1- 2: Merged signal (red and green). Green - **ab52627** observed at 110 kDa. Red - Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) observed at 37 kDa.

ab52627 was shown to react with Notch1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab261762 (knockout cell lysate **ab257006**) was used. Wild-type HeLa and NOTCH1 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. **ab52627** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) overnight at 4°C at a 1 in 1000 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.

Sanger Sequencing - Human NOTCH1 knockout HeLa cell line (ab261762)

Homozygous: 1 bp insertion in exon 5.

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