abcam

Product datasheet

Human NOTCH1 knockout HeLa cell lysate ab257006

3 图像

概述

产品名称	人NOTCH1 knockout HeLa cell裂解物
产品概述	Knockout cell lysate achieved by CRISPR/Cas9.
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)
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.*
说明	**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**.
经测试应用	**适用于:** WB

性能

存放说明	Store at -80°C. Please refer to protocols.

组件	1 kit
ab260131 - Human NOTCH1 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 vWA: 16, 18 TH01: 7 TPOX: 8, 12 CSF1PO: 9, 10

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.

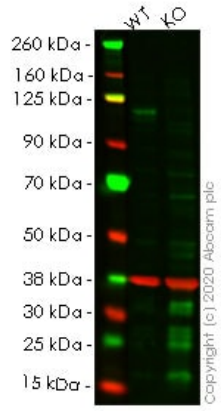
应用

The Abpromise guarantee **Abpromise™承诺保证使用ab257006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



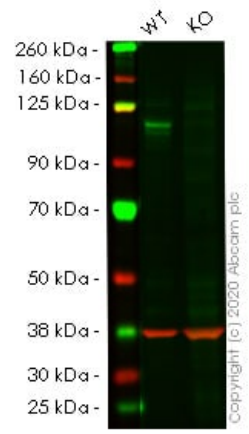
Western blot - Human NOTCH1 knockout HeLa cell lysate (ab257006)

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

Lane 2: NOTCH1 knockout HeLa cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - **ab65297** observed at 110 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab65297 Anti-Notch1 antibody was shown to specifically 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 and Notch1 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. **ab65297** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 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.



Western blot - Human NOTCH1 knockout HeLa cell lysate (ab257006)

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

Lane 2: NOTCH1 knockout HeLa cell lysate (20µg)

Lanes 1- 2: Merged signal (red and green). Green - **ab52627** observed at 110 kDa. Red - loading control **ab8245** observed at 37 kDa.

ab52627 Anti-Notch1 antibody [EP1238Y] was shown to specifically 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 and Notch1 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. **ab52627** and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated overnight at 4°C at 1 in 1000 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.

Sanger Sequencing - Human NOTCH1 knockout HeLa cell lysate (ab257006)

Homozygous: 1 bp insertion in exon 5

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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