abcam

Product datasheet

Anti-Notch1 antibody [EP1238Y] ab52627



ייבעדין RabMAb

★★★★★ 17 Abreviews 177 References 画像数 12

製品の概要

製品名 Anti-Notch1 antibody [EP1238Y]

製品の詳細 Rabbit monoclonal [EP1238Y] to Notch1

由来種 Rabbit

特異性 80% identities with Notch 2 and 81% with Notch 3

アプリケーション 適用あり: IHC-P, Flow Cyt (Intra), WB, ICC/IF

適用なし: №

種交差性 交差種: Mouse, Human

交差が予測される動物種: Cow 4

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール WB: HeLa, HAP1, MOLT-4, HEK-293 cell lysate. Mouse brain lysate. IHC-P: Human brain tissue.

ICC/IF: HeLa cells. Flow Cyt (intra): HeLa cells.

特記事項 Notch 1 (encoded by the Notch 1 gene) is a member of the Notch family of receptors. When

> bound to a ligand, the Notch1 receptor sends signals critical for normal development of many tissues throughout the body. Notch1 signalling supports the determination and specialization of

cells as well as playing a role in cell proliferation, differentiation, and apoptosis.

This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle.

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

精製度 Protein A purified

ポリ/モノ モノクローナル **クローン名** EP1238Y

アイソタイプ IgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab52627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
IHC-P	★★★★ (9)	1/100 - 1/150. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See IHC antigen retrieval protocols.
Flow Cyt (Intra)		1/130 - 1/200. ab172730 - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
WB	*** <u>*</u>	1/1000 - 1/2000. Detects a band of approximately 125 kDa.
ICC/IF		1/100 - 1/150.

追加情報

Is unsuitable for IP.

ターゲット情報

機能

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.

組織特異性

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.

関連疾患

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.

配列類似性

Belongs to the NOTCH family.
Contains 5 ANK repeats.
Contains 36 EGF-like domains.

翻訳後修飾

Contains 3 LNR (Lin/Notch) repeats.

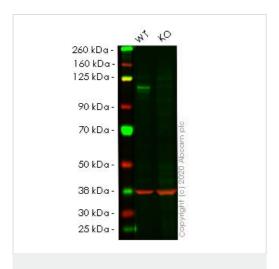
Synthesized in the endoplasmic reticulum as an inactive form which is proteolytically cleaved by a furin-like convertase in the trans-Golgi network before it reaches the plasma membrane to yield an active, ligand-accessible form. Cleavage results in a C-terminal fragment N(TM) and a N-terminal fragment 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.

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

画像

細胞内局在



Western blot - Anti-Notch1 antibody [EP1238Y] (ab52627)

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

Lane 1: Wild-type HeLa cell lysate

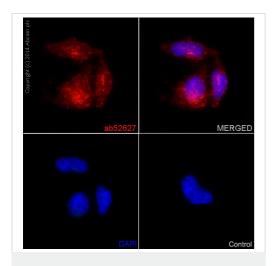
Lane 2: NOTCH1 knockout HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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.

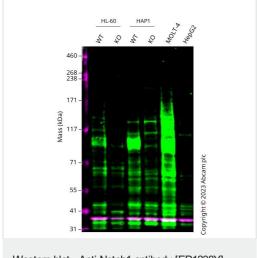
ab52627 was shown to react with Notch1 in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab261762 (knockout cell lysate ab257006) was used. Wild-type HeLa and NOTCH1 knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room 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 lgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-Notch1 antibody [EP1238Y] (ab52627)

Immunofluorescent staining of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells fixed with 4% PFA using purified ab52627 at a dilution of 1/150.

An Alexa Fluor[®] 555 goat anti-rabbit was used as the secondary and the sample was stained with DAPI. An Alexa Fluor[®] 555 goat anti-rabbit was used at a dilution of 1/500 as the **negative control** and is shown in the bottom right hand panel.



Western blot - Anti-Notch1 antibody [EP1238Y] (ab52627)

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

Lane 1: Wild-type HL-60 cell lysate

Lane 2: NOTCH1 knockout HL-60 cell lysate

Lane 3: Wild-type HAP1 cell lysate

Lane 4: NOTCH1 knockout HAP1 cell lysate

Lane 5 : MOLT-4 cell lysate

Lane 6 : HepG2 cell lysate

Lysates/proteins at 20 µg per lane.

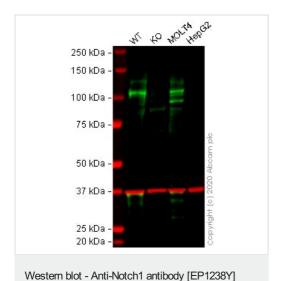
Developed using the ECL technique.

Performed under reducing conditions.

Observed band size: 100 kDa

Western blot: Anti-NOTCH1 antibody [EP1238Y] (ab52627) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab52627 was shown to bind specifically to NOTCH1. A band was observed at 100 kDa in wild-type HL-60 cell lysates with no signal observed at this size in NOTCH1 knockout cell line. To generate this image, wild-type and NOTCH1 knockout HL-60 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 lgG H&L 800CW and Goat anti-Mouse lgG H&L 680RD at 1/20000 dilution.



(ab52627)

All lanes: Anti-Notch1 antibody [EP1238Y] (ab52627) at 1 µg/ml

Lane 1: Wild-type HAP1 cell lysate at 40 µg

Lane 2: NOTCH1 knockout HAP1 cell lysate at 40 µg

Lane 3: MOLT-4 cell lysate at 20 μg **Lane 4**: HepG2 cell lysate at 20 μg

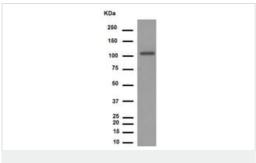
Performed under reducing conditions.

GAPDH antibody [6C5]) observed at 37kDa.

Observed band size: 105 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab52627 observed at 105 kDa. Red - loading control, <u>ab8245</u> (Mouse anti-

ab52627 was shown to react with Notch1 in wild-type HAP1 cells in western blot. Loss of signal was observed when NOTCH1 knockout sample was used. Wild-type and NOTCH1 knockout HAP1 cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk before incubation with ab52627 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at 1 µg/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-Notch1 antibody [EP1238Y] (ab52627)

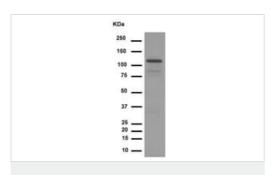
Anti-Notch1 antibody [EP1238Y] (ab52627) at 1/2000 dilution (Purified) + Mouse brain at $10 \mu g$

Secondary

HRP goat anti-rabbit (H+L) at 1/1000 dilution

Observed band size: 125 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-Notch1 antibody [EP1238Y] (ab52627)

Anti-Notch1 antibody [EP1238Y] (ab52627) at 1/2000 dilution (Purified) + HEK-293 (Human epithelial cell line from embryonic kidney) cell lysate at 10 µg

Secondary

HRP goat anti-rabbit (H+L) at 1/1000 dilution

Observed band size: 125 kDa

B MeWo Luc

s.c. tumor

lung
metastasis

NOTCH1

MeWo L1 kd

s.c. tumor

lung
metastasis

Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Notch1 antibody
[EP1238Y] (ab52627)

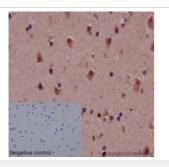
Ernst et al PLoS One. 2018 Feb 12;13(2):e0192525. doi: 10.1371/journal.pone.0192525. eCollection 2018. Fig 5. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

Blocking/Dilution buffer: 5% NFDM/TBST.

Verification of gene expression array data by immunohistochemical analysis of Notch 1 expression in subcutaneous tumors and lung metastases from a human melanoma (MeWo) xenograft experiment in mice.

Immunohistochemical staining for Notch1 expression (ab52627, red) in subcutaneous tumors and lung metastases (both panels) of MeWo (Human malignant melanoma cell line) cells.

All scale bars: 50 µm.

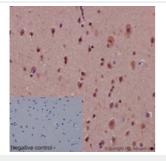


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Notch1 antibody
[EP1238Y] (ab52627)

Immunohistochemical staining of paraffin-embedded human brain with purified ab52627 at a dilution of 1/150.

A prediluted HRP polymer for rabbit IgG was used as the secondary and the sample was stained with hematoxylin. PBS was used instead of the primary antibody as the **negative control**, and is shown in the inset.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



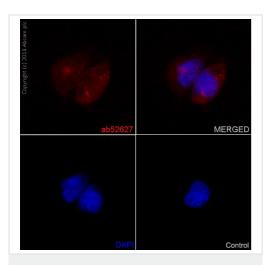
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Notch1 antibody

[EP1238Y] (ab52627)

Immunohistochemical staining of paraffin-embedded human brain with unpurified ab52627 at a dilution of 1/100.

A prediluted HRP polymer for rabbit lgG was used as the secondary and the sample was stained with hematoxylin. PBS was used instead of the primary antibody as the **negative control**, and is shown in the inset.

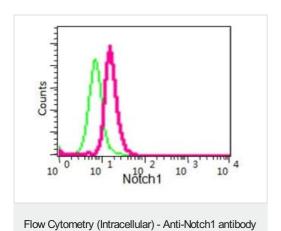
Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-Notch1 antibody [EP1238Y] (ab52627)

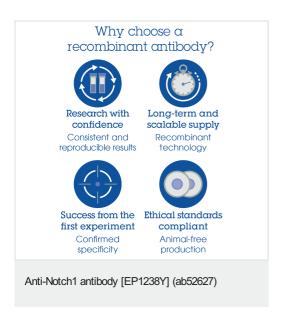
Immunofluorescent staining of HeLa (Human epithelial cell line from cervix adenocarcinoma) cells fixed with 4% PFA using unpurified ab52627 at a dilution of 1/100.

An Alexa Fluor[®] 555 goat anti-rabbit was used as the secondary and the sample was stained with DAPI. An Alexa Fluor[®] 555 goat anti-rabbit was used at a dilution of 1/500 as the **negative control** and is shown in the bottom right hand panel.



[EP1238Y] (ab52627)

Intracellular Flow Cytometry analysis of permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells (2% PFA, pink) withpurified ab52627 at a 1/200 dilution, or **negative control** rabbit monoclonal lgG (green). The secondary antibody was FITC goat anti-rabbit.



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