abcam

Product datasheet

Anti-ENT1 antibody [SP120] ab182023





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Overview

Product name Anti-ENT1 antibody [SP120]

Description Rabbit monoclonal [SP120] to ENT1

Host species Rabbit

Tested applications Suitable for: IHC-P, WB, ICC, Flow Cyt (Intra)

Species reactivity Reacts with: Human

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

Positive control IHC-P: Human Pancreas, Human tonsil, Human pancreas, and Human colon carcinoma tissue;

Flow Cyt (Intra): HepG2 cells; ICC: HAP1 cells; WB: HAP1 cell lysate.

General notes 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.

This product is FOR RESEARCH USE ONLY. For commercial use, please contact

partnerships@abcam.com.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.60

> Preservative: 0.1% Sodium azide Constituents: PBS, 1% BSA

Purity Protein A/G purified

Purification notes Purified from TCS by protein A/G.

Clonality Monoclonal

Clone number SP120

Isotype IgG

Applications

The Abpromise guarantee

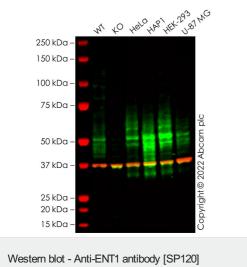
Our <u>Abpromise guarantee</u> covers the use of ab182023 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-P		1/100. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Predicted molecular weight: 50 kDa.
ICC		1/200.
Flow Cyt (Intra)		Use at an assay dependent concentration.

Target		
Function	Mediates both influx and efflux of nucleosides across the membrane (equilibrative transporter). It is sensitive (ES) to low concentrations of the inhibitor nitrobenzylmercaptopurine riboside (NBMPR) and is sodium-independent. It has a higher affinity for adenosine. Inhibited by dipyridamole and dilazep (anticancer chemotherapeutics drugs).	
Tissue specificity	Expressed in heart, brain, mammary gland, erythrocytes and placenta, and also in fetal liver and spleen.	
Sequence similarities	Belongs to the SLC29A transporter family.	
Post-translational modifications	Glycosylated.	
Cellular localization	Basolateral cell membrane. Apical cell membrane. Predominantly localized in the basolateral membrane in polarised MDCK cells.	

Images



Western blot - Anti-ENT1 antibody [SP120] (ab182023)

All lanes: Anti-ENT1 antibody [SP120] (ab182023) at 1/1000

dilution

Lane 1: Wild-type HEK-293T cell lysate

Lane 2: SLC29A1 knockout HEK-293T cell lysate

Lane 3 : HeLa cell lysate

Lane 4 : HAP1 cell lysate

Lane 5 : HEK-293 cell lysate

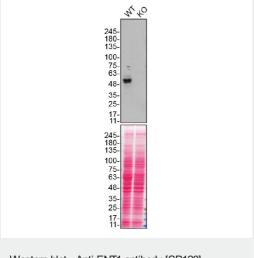
Lane 6 : U-87 MG cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 50 kDa **Observed band size:** 40-60 kDa

False colour image of Western blot: Anti-ENT1 antibody [SP120] staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in red. In Western blot, ab182023 was shown to bind specifically to ENT1. A band was observed at 40-60 kDa in wildtype HEK-293T cell lysates with no signal observed at this size in SLC29A1 knockout cell line. To generate this image, wild-type and SLC29A1 knockout HEK-293T 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 800CW and Goat anti-Mouse IgG H&L 680RD at 1/20000 dilution.



Western blot - Anti-ENT1 antibody [SP120] (ab182023)

All lanes : Anti-ENT1 antibody [SP120] (ab182023) at 1/1000 dilution

Lane 1: Wild-type HAP1 cell lysate

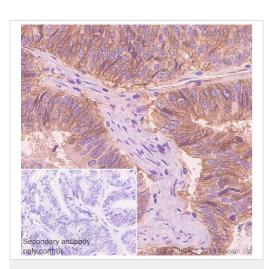
Lane 2: SLC29A1 knockout HAP1 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

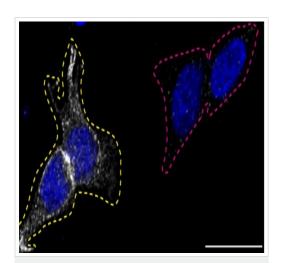
Predicted band size: 50 kDa

ab182023 was shown to react with SLC29A1 in wild-type HAP1 cells in Western blot with loss of signal observed in a SLC29A1 knockout cell line. Wild-type HAP1 and SLC29A1 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab182023 overnight at 4 °C at a 1/1000 dilution. Blots were incubated with goat antirabbit HRP secondary antibodies at 0.2ug/mL before imaging. These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ENT1 antibody [SP120] (ab182023)

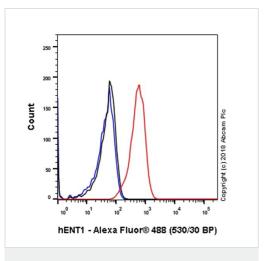
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human colon carcinoma tissue sections labeling ENT1 with Purified ab182023 at 1/100 dilution (0.77 µg/ml). Heat mediated antigen retrieval was performed Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20mins. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunocytochemistry - Anti-ENT1 antibody [SP120] (ab182023)

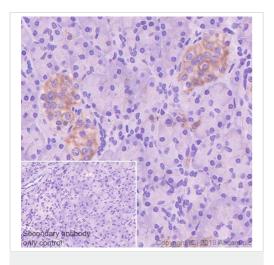
ab182023 was shown to react with SLC29A1 in wild-type HAP1 cells in Immunocytochemistry with loss of signal observed in a SLC29A1 knockout cell line. Wild-type and knockout cells were mixed and pelleted at a 1:1 ratio on coverslips. The cells were fixed with 4% paraformaldehyde (15 min) then permeabilized with 0.1% Triton X-100 (10min) and then blocked with . The cells were then incubated with ab182023 at 1/200 dilution overnight at 4°C followed by a further incubation at room temperature for 1h with a goat antirabbit secondary antibody to (Alexa Fluor® 555) at 0.5 µg/ml. Acquisition of the green (wild-type), red (antibody staining) and farred (knockout) channels was performed. Representative grayscale images of the red channel are shown. Wild-type and knockout cells are outlined with yellow and magenta dashed line, respectively. Schematic representation of the mosaic strategy used is shown on the bottom-right panel. Image was acquired with a Zeiss(LSM-880). These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially

available antibodies.



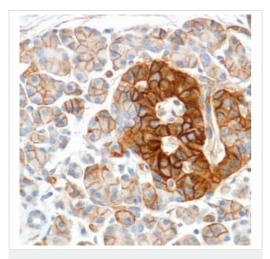
Flow Cytometry (Intracellular) - Anti-ENT1 antibody [SP120] (ab182023)

Flow Cytometry analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling ENT1 with purified ab182023 at (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit lgG (Alexa Fluor[®] 488, <u>ab150077</u>) was used as the secondary antibody. Isotype control - Rabbit monoclonal lgG (<u>ab172730</u>) / Black. Unlabeled control - Unlabelled cells / blue.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ENT1 antibody [SP120] (ab182023)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human pancreas tissue sections labeling ENT1 with Purified ab182023 at 1/100 dilution (0.77 µg/ml). Heat mediated antigen retrieval was performed Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20mins. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ENT1 antibody [SP120] (ab182023)

Why choose a

Immunohistochemical analysis of formalin-fixed paraffin-embedded Human pancreas tissue labeling ENT1 with ab182023.







Anti-ENT1 antibody [SP120] (ab182023)

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