# abcam

### Product datasheet

## Anti-ALDH1A1 antibody [EP1933Y] - C-terminal ab52492



יוכציטו RabMAb

★★★★★ 22 Abreviews 170 References 画像数 14

製品の概要

Anti-ALDH1A1 antibody [EP1933Y] - C-terminal 製品名

製品の詳細 Rabbit monoclonal [EP1933Y] to ALDH1A1 - C-terminal

由来種 Rabbit

特異性 The mouse recommendation is based on the WB results. We do not guarantee IHC-P for mouse.

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

種交差性 交差種: Mouse, Human

免疫原 Synthetic peptide within Human ALDH1A1 aa 300-400 (C terminal). The exact sequence is

proprietary.

Database link: P00352

ポジティブ・コントロール WB: HepG2 and NIH/3T3 cell lysate; Human small intestine and mouse liver tissue lysate. IHC:

Human liver, kidney, lung and brain tissue, human breast carcinoma. ICC/IF: HepG2 cells. Flow

Cyt (intra): HepG2 cells. IP: HepG2 cell lysate

特記事項 The mouse recommendation is based on the WB results. We do not guarantee IHC-P for

mouse.

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**.

Rat: We have preliminary internal testing data to indicate this antibody may not react with this

species. Please contact us for more information.

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 long

term. Avoid freeze / thaw cycle.

**バッファー** pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol, 0.05% BSA

精製度 Protein A purified

**ポリモ**ノクローナル **ウローン名** EP1933Y

アイソタイプ lgG

#### 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ab52492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
Flow Cyt (Intra)		1/20. For unpurified use at 1/1000. <u>ab172730</u> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF	<b>★★★★★ (3)</b>	1/100 - 1/250.
WB	<b>★★★★ (11)</b>	1/500 - 1/2000. Detects a band of approximately 55 kDa (predicted molecular weight: 55 kDa).
IP		1/20 - 1/30.
IHC-P	<b>★★★</b> ★ (5)	1/50. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. See <b>IHC antigen retrieval protocols</b> .  The mouse recommendation is based on the WB results. We do not guarantee IHC-P for mouse.

#### ターゲット情報

機能 Binds free retinal and cellular retinol-binding protein-bound retinal. Can convert/oxidize

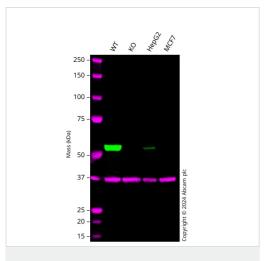
retinaldehyde to retinoic acid.

パスウェイ Cofactor metabolism; retinol metabolism.

**配列類似性** Belongs to the aldehyde dehydrogenase family.

細胞内局在 Cytoplasm.

## 画像



Western blot - Anti-ALDH1A1 antibody [EP1933Y] - C-terminal (ab52492)

**All lanes :** Anti-ALDH1A1 antibody [EP1933Y] - C-terminal (ab52492) at 1/1000 dilution

Lane 1: Wild-type A549 cell lysate

Lane 2: ALDH1A1 knockout A549 cell lysate

Lane 3 : HepG2 cell lysate

Lane 4 : MCF7 cell lysate

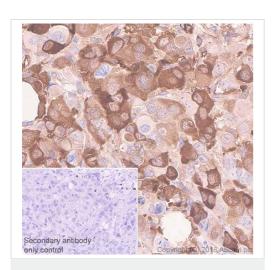
Lysates/proteins at 20 µg per lane.

#### **Secondary**

**All lanes :** Goat anti-Rabbit lgG H&L 800CW and Goat anti-Mouse lgG H&L 680RD at 1/20000 dilution

**Predicted band size:** 55 kDa **Observed band size:** 55 kDa

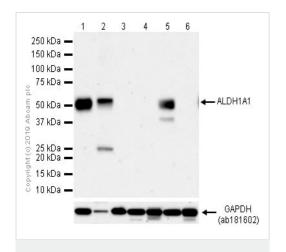
Western blot: Anti-ALDH1A1 antibody [EP1933Y] (ab52492) staining at 1/1000 dilution, shown in green; Mouse anti-GAPDH antibody [6C5] (ab8245) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab52492 was shown to bind specifically to ALDH1A1. A band was observed at 55 kDa in wild-type A549 cell lysates with no signal observed at this size in ALDH1A1 knockout cell line. To generate this image, wild-type and ALDH1A1 knockout A549 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.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ALDH1A1 antibody
[EP1933Y] - C-terminal (ab52492)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human breast carcinoma tissue sections labeling ALDH1A1 with purified ab52492 at 1/50 dilution (3.54 µg/ml). Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody.

Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-ALDH1A1 antibody [EP1933Y] - C-terminal (ab52492)

**All lanes :** Anti-ALDH1A1 antibody [EP1933Y] - C-terminal (ab52492) at 1/1000 dilution (Purified)

**Lane 1 :** HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates at 20  $\mu g$ 

Lane 2: Human small intestine lysates

Lane 3 : PC-3 (Human prostate adenocarcinoma epithelial cell) whole cell lysates at 20 µg

**Lane 4 :** MCF-7 (Human breast adenocarcinoma epithelial cell) whole cell lysates at 20 μg

Lane 5: Mouse liver lysates at 20 µg

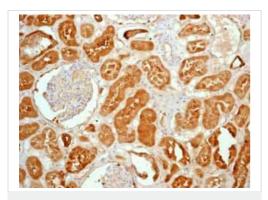
**Lane 6 :** NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates at  $20 \ \mu g$ 

#### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

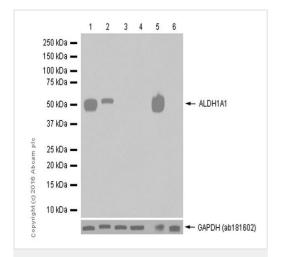
Predicted band size: 55 kDa

Observed band size: 55 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ALDH1A1 antibody
[EP1933Y] - C-terminal (ab52492)

Immunohistochemical analysis of Formalin/PFA-fixed paraffinembedded normal human kidney tissue sections labeling ALDH1A1 with ab52492 (unpurified).



Western blot - Anti-ALDH1A1 antibody [EP1933Y] - C-terminal (ab52492)

**All lanes :** Anti-ALDH1A1 antibody [EP1933Y] - C-terminal (ab52492) at 1/1000 dilution (unpurified)

**Lane 1 :** HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysate

Lane 2: Human small intestine tissue lysate

**Lane 3 :** PC-3 (Human prostate adenocarcinoma epithelial cell) whole cell lysate

**Lane 4 :** MCF-7 (Human breast adenocarcinoma epithelial cell) whole cell lysate

Lane 5: Mouse liver tissue lysate

Lane 6: NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate

Lysates/proteins at 15 µg per lane.

#### Secondary

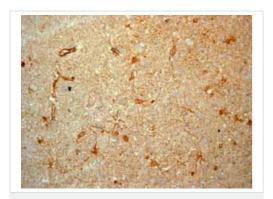
**All lanes :** Anti-Rabbit lgG (HRP), specific to the non-reduced form of lgG at 1/2000 dilution

Predicted band size: 55 kDa

Observed band size: 55 kDa

Exposure time: 1 minute

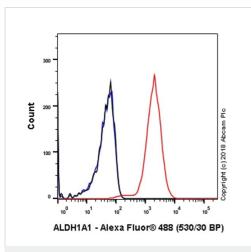
Blocking/Diluting buffer 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ALDH1A1 antibody

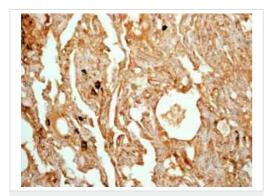
[EP1933Y] - C-terminal (ab52492)

Immunohistochemical analysis of Formalin/PFA-fixed paraffinembedded normal human brain tissue sections labeling ALDH1A1 with ab52492 (unpurified).



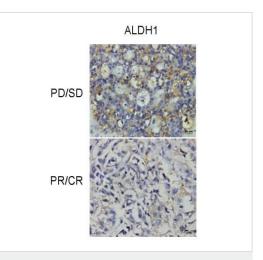
Flow Cytometry (Intracellular) - Anti-ALDH1A1 antibody [EP1933Y] - C-terminal (ab52492)

Intracellular Flow Cytometry analysis of HepG2 (Human hepatocellular carcinoma epithelial cell) cells labeling ALDH1A1 with purified ab52492 at 1/20 dilution (10µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluorr® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ALDH1A1 antibody
[EP1933Y] - C-terminal (ab52492)

Immunohistochemical analysis of Formalin/PFA-fixed paraffinembedded normal human lung tissue sections labeling ALDH1A1 with ab52492 (unpurified).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ALDH1A1 antibody
[EP1933Y] - C-terminal (ab52492)

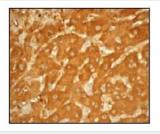
Gong et al PLoS One. 2010 Dec 20;5(12):e15630. doi: 10.1371/journal.pone.0015630. Fig 1. Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

Tumor tissues of primary invasive ductal carcinomas of the breast were obtained from 192 female patients with stage IIB and III prior to pre-operative neoadjuvant chemotherapy.

(progressive or stable disease, PD/SD)

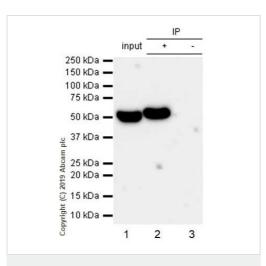
(partial or complete remission, PR/CR)

The level of ALDH1 was tested by immunohistochemistry staining in paraffin-embedded tissue sections. Rabbit monoclonal ALDH1A1 antibody (unpurified ab52492, Abcam) used at a 1:100 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ALDH1A1 antibody
[EP1933Y] - C-terminal (ab52492)

Immunohistochemical analysis of paraffin-embedded human liver tissue sections labeling ALDH1A1 with ab52492 (unpurified) at 1/100 dilution.



Immunoprecipitation - Anti-ALDH1A1 antibody [EP1933Y] - C-terminal (ab52492) ab52492 (purified) at 1/20 dilution (2µg) immunoprecipitating ALDH1A1 in HepG2 (Human hepatocellular carcinoma epithelial cell) whole cell lysates.

Lane 1: HepG2 whole cell lysates 10µg.

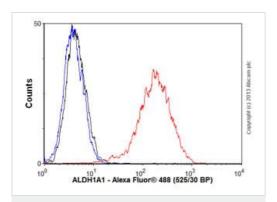
Lane 2: ab52492 IP in HepG2 whole cell lysates.

 $\label{lambda} \textbf{Lane 3:} \ \ \text{Rabbit monoclonal lgG } (\underline{\textbf{ab172730}}) \ \ \text{instead of ab52492 in} \\ \ \ \text{HepG2 whole cell lysates}.$ 

For western blotting, VeriBlot for IP Detection Reagent (HRP)

(ab131366) was used at 1/1000 dilution.

Blocking and diluting buffer: 5% NFDM/TBST.

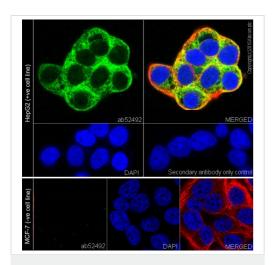


Flow Cytometry (Intracellular) - Anti-ALDH1A1 antibody [EP1933Y] - C-terminal (ab52492)

Overlay histogram showing HepG2 (Human liver hepatocellular carcinoma cell line) cells stained with ab52492 (unpurified) (red line).

The cells were fixed with 80% methanol (5 minutes) and then permeabilized with 0.1% PBS-Tween for 20 minutes. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab52492, 1/1000 dilution) for 30 minutes at 22°C. The secondary antibody used was Alexa Fluorr® 488 goat anti-rabbit lgG (H&L) (ab150077) at 1/2000 dilution for 30 minutes at 22°C. Isotype control antibody (black line) was rabbit lgG (monoclonal) (0.1 $\mu$ g/1x10<sup>6</sup> cells) used under the same conditions. Unlabeled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.



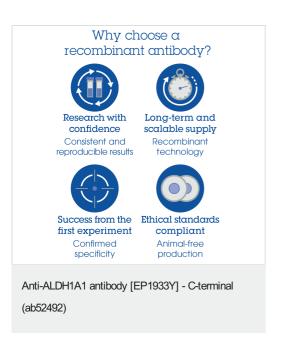
Immunocytochemistry/ Immunofluorescence - Anti-ALDH1A1 antibody [EP1933Y] - C-terminal (ab52492)

Immunocytochemistry/ Immunofluorescence analysis of HepG2 (Human liver hepatocellular carcinoma cell line) cells labeling ALDH1A1 with ab52492 (purified) at 1/500 dilution (4 µg/ml).

Cells were fixed in 100% methanol. <a href="mailto:ab150077">ab150077</a>, an AlexaFluor<sup>®</sup>488 Goat anti-Rabbit secondary antibody was used at 1/1000 dilution (2 µg/ml). <a href="mailto:ab195889">ab195889</a>, Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) was used to counterstain at 1/200 dilution (2.5 µg/ml). DAPI was used as nuclear counterstain.

Confocal image showing cytoplasmic staining on HepG2 cell line.

Negative control: No staining on MCF-7 cell line.



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