# abcam

# **Product datasheet**

# Anti-ALKBH5 antibody [EPR18958] ab195377

敲除 验证 重组 RabMAb

★★★★★ <u>4 Abreviews</u> <u>51 References</u> 6 图像

# 概述

Anti-ALKBH5 <b>抗体</b> [EPR18958]
兔单克隆抗体[EPR18958] to ALKBH5
Rabbit
This antibody has been tested in IHC-P on Human, Mouse and Rat testis sections. It worked well on Human, but not on the Mouse or Rat sections, under our experimental conditions.
适用于: WB, IHC-P
与反应: Mouse, Rat, Human
预测可用于: Sheep, Goat, Horse, Guinea pig, Cow, Cat, Dog, Pig, Non human primates 🛛 🔺
Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
WB: Wild type MEF, HeLa, HepG2 , PC-12, NIH/3T3, Jurkat, Neuro-2a, HEK-293T and F9 whole cell lysates. IHC-P: Human testis and kidney tissues.
<ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <li>High batch-to-batch consistency and reproducibility</li> <li>Improved sensitivity and specificity</li> <li>Long-term security of supply</li> <li>Animal-free production</li> <li>For more information <u>see here</u>.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u>.</li> </ul>

性能	
形式	Liquid
存 <b>放</b> 说明	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.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
纯 <b>度</b>	Protein A purified

克隆	单 <b>克隆</b>
<b>克隆</b> 编号	EPR18958
同种型	lgG

应用

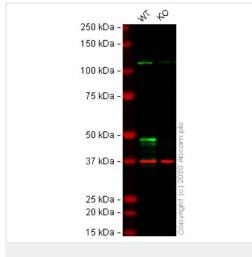
# The Abpromise guarantee \_承诺保证使用ab195377于以下的经测试应用

"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	★ ★ ★ ★ ★ <u>(2)</u>	1/1000. Detects a band of approximately 35-44 kDa (predicted molecular weight: 44 kDa).
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. Suitable for human tissues only.

<b>靶</b> 标	
相关性	Dioxygenase that demethylates RNA by oxidative demethylation: specifically demethylates N(6)- methyladenosine (m6A) RNA, the most prevalent internal modification of messenger RNA (mRNA) in higher eukaryotes. Requires molecular oxygen, alpha-ketoglutarate and iron. Demethylation of m6A mRNA affects mRNA processing and export and is required for spermatogenesis.
细 <b>胞定位</b>	Nucleus speckle

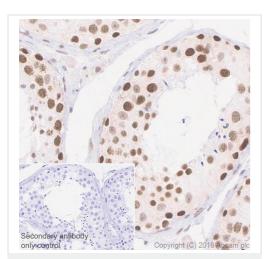
图片





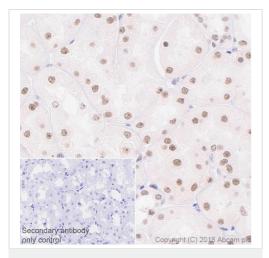
All lanes : Anti-ALKBH5 antibody [EPR18958] (ab195377) at 1/1000 dilution Lane 1 : Wild-type HEK-293T cell lysate Lane 2 : ALKBH5 knockout HEK-293T cell lysate Lysates/proteins at 20 µg per lane. Performed under reducing conditions. Predicted band size: 44 kDa Observed band size: 48 kDa Lanes 1 - 2: Merged signal (red and green). Green - ab195377 observed at 48 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab195377 was shown to react with ALKBH5 in wild-type HEK-293T cells in western blot with loss of signal observed in ALKBH5 knockout cell line **ab266762** (ALKBH5 knockout cell lysate **ab257349**). Wild-type and ALKBH5 knockout HEK-293T cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% milk in TBS-T (0.1% Tween<sup>®</sup>) before incubation with ab195377 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 800CW) preabsorbed (**ab216772**) and Goat anti-Rabbit IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed (**ab216777**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



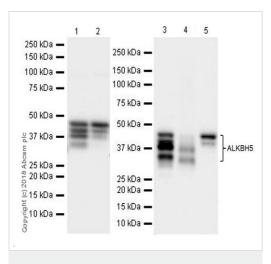
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ALKBH5 antibody [EPR18958] (ab195377) Immunohistochemical analysis of paraffin-embedded human testis tissue labeling ALKBH5 with ab195377 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear staining in human testis (PMID: 21264265; PMID: 27590511; PMID: 17979886) is observed. Counter stained with hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-ALKBH5 antibody [EPR18958] (ab195377) Immunohistochemical analysis of paraffin-embedded human kidney tissue labeling ALKBH5 with ab195377 at 1/2000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Nuclear staining in human kidney (PMID: 21264265) is observed. Counter stained with hematoxylin. Heat mediated antigen retrieval was performed using Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit IgG H&L (HRP) Ready to use.



Western blot - Anti-ALKBH5 antibody [EPR18958] (ab195377) Lanes 1-2 : Anti-ALKBH5 antibody [EPR18958] (ab195377) at 1/1000 dilution

Lanes 3-5 : Anti-ALKBH5 antibody [EPR18958] (ab195377) at 1/2000 dilution

Lane 1 : PC-12 (rat adrenal gland pheochromocytoma cell line) whole cell lysate

Lane 2 : NIH/3T3 (mouse embyro fibroblast cell line) whole cell lysate

Lane 3 : Jurkat (human T cell leukemia cell line from peripheral blood) whole cell lysate

Lane 4 : Neuro-2a (mouse neuroblastoma cell line) whole cell lysate

Lane 5 : F9 (mouse embryonic testicular cancer cell line) whole cell lysate

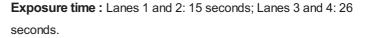
Lysates/proteins at 10 µg per lane.

#### Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

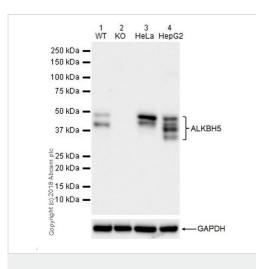
Developed using the ECL technique.

Predicted band size: 44 kDa Observed band size: 35-44 kDa



Blocking/Dilution buffer: 5% NFDM/TBST.

The molecular mass observed is consistent with the literature (PMID 21264265).



Western blot - Anti-ALKBH5 antibody [EPR18958] (ab195377) All lanes : Anti-ALKBH5 antibody [EPR18958] (ab195377) at 1/5000 dilution

Lane 1 : Wild type MEF whole cell lysate

Lane 2 : ALKBH5 knockout MEF whole cell lysate

Lane 3 : HeLa (human epithelial cell line from cervix

adenocarcinoma) whole cell lysate

Lane 4 : HepG2 (human liver hepatocellular carcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

# Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Developed using the ECL technique.

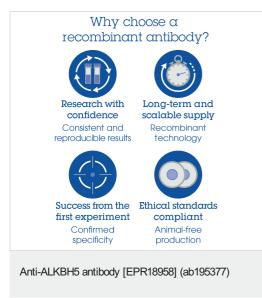
Predicted band size: 44 kDa Observed band size: 35-44 kDa

Exposure time: 70 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The Wt and ALKBH5 KO cell lysates were kindly provided by an anonymous collaborator.

The molecular mass observed is consistent with the literature (PMID 21264265).



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