abcam

Product datasheet

Anti-ALKBH1 antibody [EPR19215] ab195376





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Overview

Product name Anti-ALKBH1 antibody [EPR19215]

Description Rabbit monoclonal [EPR19215] to ALKBH1

Host species Rabbit

Tested applications Suitable for: WB, IP

Unsuitable for: ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Sheep, Goat, Horse, Guinea pig, Cow, Cat, Dog, Non human primates

Immunogen Recombinant full length protein. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Jurkat, K562, A549, A431, C6, RAW 264.7, PC-12 and NIH/3T3 whole cell lysates; human

fetal kidney, colon and fetal liver lysates; mouse brain and spleen lysates; rat spleen lysate. IP:

K562 whole 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.

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

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: 7.2

Preservative: 0.01% Sodium azide

Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal
Clone number EPR19215

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab195376 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
WB	****(1)	1/1000. Detects a band of approximately 44 kDa (predicted molecular weight: 44 kDa). Milk recommended as blocking agent.
IP		1/30.

Application notes Is unsuitable for ICC/IF.

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Function Dioxygenase that repairs alkylated single-stranded DNA and RNA containing 3-methylcytosine by

oxidative demethylation. Requires molecular oxygen, alpha-ketoglutarate and iron. May have a role in placental trophoblast lineage differentiation (By similarity). Has DNA lyase activity and introduces double-stranded breaks at abasic sites. Cleaves both single-stranded DNA and double-stranded DNA at abasic sites, with the greatest activity towards double-stranded DNA with two abasic sites. DNA lyase activity does not require alpha-ketoglutarate and iron.

Tissue specificity Ubiquitous.

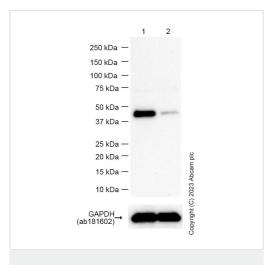
Sequence similarities Belongs to the alkB family.

Contains 1 Fe2OG dioxygenase domain.

Cellular localization Mitochondrion. Nucleus. Mainly localizes in euchromatin, largely excluded from heterochromatin

and nucleoli.

Images



Western blot - Anti-ALKBH1 antibody [EPR19215] (ab195376)

All lanes : Anti-ALKBH1 antibody [EPR19215] (ab195376) at 1/1000 dilution

Lane 1: A549 (Human lung carcinoma epithelial cell) transfected with scrambled siRNA control whole cell lysate

Lane 2: A549 (Human lung carcinoma epithelial cell) transfected with siRNA specifically targeting ALKBH1 whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution (Goat Anti-Rabbit lgG, (H+L), Peroxidase conjugated)

Predicted band size: 44 kDa **Observed band size:** 44 kDa

Exposure time: 120 seconds

Blocking and diluting buffer: 5% NFDM/TBST

260 kDa 160 kDa 125 kDa 90 kDa 70 kDa 38 kDa 30 kDa 25 kDa 15 kDa -

Western blot - Anti-ALKBH1 antibody [EPR19215] (ab195376)

All lanes : Anti-ALKBH1 antibody [EPR19215] (ab195376) at 1/1000 dilution

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: ALKBH1 knockout HAP1 whole cell lysate

Lane 3: A549 whole cell lysate

Lysates/proteins at 20 µg per lane.

Predicted band size: 44 kDa

Lanes 1 - 3: Merged signal (red and green). Green - ab195376 observed at 43 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

ab195376 was shown to specifically react with in wild-type HAP1 cells as signal was lost in ALKBH1 knockout cells. Wild-type and ALKBH1 knockout samples were subjected to SDS-PAGE. The membrane was blocked with 3% Milk. Ab195376 and ab8245 (Mouse anti-GAPDH loading control) were incubated overnight at

4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed <u>ab216773</u> and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed <u>ab216776</u> secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.

1 2 3 4

250 kDa —

150 kDa —

100 kDa —

75 kDa —

37 kDa —

25 kDa —

20 kDa —

10 kDa —

10 kDa —

10 kDa —

Western blot - Anti-ALKBH1 antibody [EPR19215] (ab195376)

All lanes : Anti-ALKBH1 antibody [EPR19215] (ab195376) at 1/1000 dilution

Lane 1 : Jurkat (Human T cell leukemia cell line from peripheral blood) whole cell lysate

Lane 2: K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate

Lane 3 : A549 (Human lung carcinoma cell line) whole cell lysate

Lane 4 : A431 (Human epidermoid carcinoma cell line) whole cell
lysate

Lysates/proteins at 10 µg per lane.

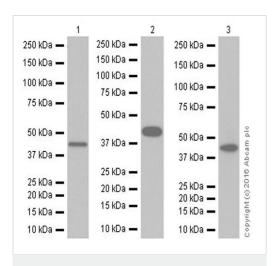
Secondary

All lanes : Goat Anti-Rabbit $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at 1/100000 dilution

Predicted band size: 44 kDa **Observed band size:** 44 kDa

Exposure time: 30 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-ALKBH1 antibody [EPR19215] (ab195376)

All lanes : Anti-ALKBH1 antibody [EPR19215] (ab195376) at 1/1000 dilution

Lane 1: Human fetal kidney lysate

Lane 2: Human colon lysate

Lane 3: Human fetal liver lysate

Lysates/proteins at 10 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

Predicted band size: 44 kDa **Observed band size:** 44 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

1 2 3 4 250 kDa -250 kDa -250 kDa -250 kDa -150 kDa -150 kDa -150 kDa -150 kDa -100 kDa -100 kDa -100 kDa -100 kDa -75 kDa 🕳 75 kDa -75 kDa -75 kDa -50 kDa -50 kDa -50 kDa -50 kDa -37 kDa -37 kDa -37 kDa -37 kDa -25 kDa -25 kDa -25 kDa -25 kDa -20 kDa -20 kDa -20 kDa -20 kDa -15 kDa -15 kDa -15 kDa -15 kDa -10 kDa -10 kDa -10 kDa -10 kDa -

Western blot - Anti-ALKBH1 antibody [EPR19215] (ab195376)

All lanes : Anti-ALKBH1 antibody [EPR19215] (ab195376) at 1/1000 dilution

Lane 1: C6 (Rat glial tumor cell line) whole cell lysate

Lane 2: RAW 264.7 (Mouse macrophage cell line transformed

with Abelson murine leukemia virus) whole cell lysate

Lane 3: PC-12 (Rat adrenal gland pheochromocytoma cell line)

whole cell lysate

Lane 4: NIH/3T3 (Mouse embryonic fibroblast cell line) whole cell

lysate

Lane 5: Mouse brain lysate

Lane 6: Mouse spleen lysate

Lane 7: Rat spleen lysate

Lysates/proteins at 10 µg per lane.

Secondary

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

Predicted band size: 44 kDa **Observed band size:** 44 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time: Lane 1-4: 30 seconds Lane 5-7: 3 minutes.

ALKBH1 was immunoprecipitated from 1mg of K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate with ab195376 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab195376 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

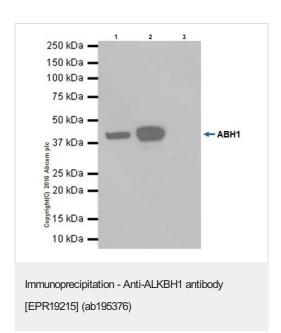
Lane 1: K562 whole cell lysate 10µg (Input).

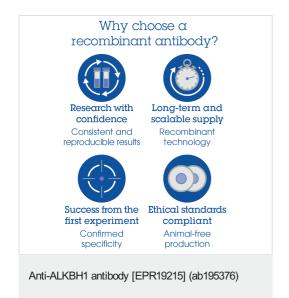
Lane 2: ab195376 IP in K562 whole cell lysate.

Lane 3: Rabbit monoclonal lgG ($\underline{ab172730}$) instead of ab195376 in K562 whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds.





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