abcam

Product datasheet

Anti-SMYD3 antibody [EPR19311] ab199361





1 References 7 Images

Overview

Product name Anti-SMYD3 antibody [EPR19311]

Rabbit monoclonal [EPR19311] to SMYD3 **Description**

Host species Rabbit

Tested applications Suitable for: WB, IP

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Human fetal kidney and fetal brain lysates; wild-type HAP1, HeLa, MCF7, HEK-293, T-47D,

HCT 116, LLC, C6, RAW 264.7, PC-12 and NIH/3T3 whole cell lysates; Mouse brain, kidney and

spleen lysates; Rat brain and kidney lysates. IP: 293T 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 EPR19311

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab199361 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		1/2000. Detects a band of approximately 45 kDa (predicted molecular weight: 49 kDa).
IP		1/50.

Target

Function	Histone methyltransferase. Specifically methylates 'Lys-4' of histone H3, inducing di- and tri-
	methylation, but not monomethylation. Plays an important role in transcriptional activation as a

member of an RNA polymerase complex. Binds DNA containing 5'-CCCTCC-3' or 5'-GAGGGG-

3' sequences.

Tissue specificity Expressed in skeletal muscles and testis. Overexpressed in a majority of colorectal and

hepatocellular carcinomas.

Sequence similaritiesBelongs to the histone-lysine methyltransferase family.

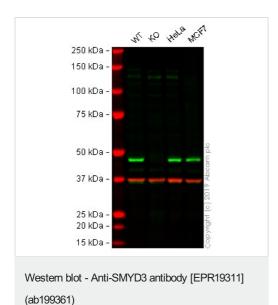
Contains 1 MYND-type zinc finger.

Contains 1 SET domain.

Cytoplasm. Nucleus. Mainly cytoplasmic when cells are arrested at G0/G1. Accumulates in the

nucleus at S phase and G2/M.

Images



All lanes: Anti-SMYD3 antibody [EPR19311] (ab199361) at

1/1000 dilution

Lane 1: Wild-type HAP1 whole cell lysate

Lane 2: SMYD3 knockout HAP1 whole cell lysate

Lane 3: HeLa whole cell lysate

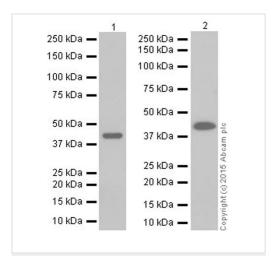
Lane 4: MCF7 whole cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 49 kDa Observed band size: 49 kDa **Lanes 1 - 4:** Merged signal (red and green). Green - ab199361 observed at 49 kDa. Red - loading control, <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37kDa.

ab199361 was shown to react with SMYD3 in HAP1 wild-type cells in Western blot. Loss of signal was observed when SMYD3 knockout sample was used. HAP1 wild-type and SMYD3 knockout whole cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3% Milk in TBS-T (0.1% Tween®) before incubation with ab199361 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 developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Western blot - Anti-SMYD3 antibody [EPR19311] (ab199361)

All lanes : Anti-SMYD3 antibody [EPR19311] (ab199361) at 1/2000 dilution

Lane 1: Human fetal kidney lysate

Lane 2: Human fetal brain lysate

Lysates/proteins at 10 µg per lane.

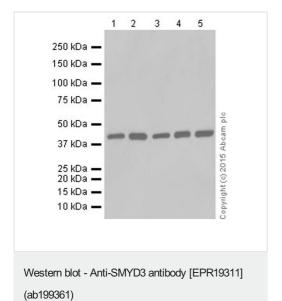
Secondary

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

Predicted band size: 49 kDa **Observed band size:** 45 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



All lanes : Anti-SMYD3 antibody [EPR19311] (ab199361) at 1/2000 dilution

Lane 1 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate

Lane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 3 : T-47D (Human ductal breast epithelial tumor cell line) whole cell lysate

Lane 4: HCT 116 (Human colorectal carcinoma cell line) whole cell lysate

Lane 5: LLC (Mouse lung carcinoma cell line) whole cell lysate

Lysates/proteins at 20 µg per lane.

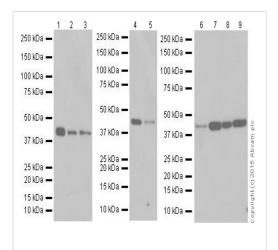
Secondary

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

Predicted band size: 49 kDa **Observed band size:** 45 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-SMYD3 antibody [EPR19311] (ab199361)

All lanes : Anti-SMYD3 antibody [EPR19311] (ab199361) at 1/2000 dilution

Lane 1: Mouse brain lysate

Lane 2: Mouse kidney lysate

Lane 3: Mouse spleen lysate

Lane 4: Rat brain lysate

Lane 5: Rat kidney lysate

Lane 6: C6 (Rat glial tumor cell line) whole cell lysate

Lane 7: RAW 264.7 (Mouse macrophage cell line transformed

with Abelson murine leukemia virus) whole cell lysate

Lane 8 : PC-12 (Rat adrenal gland pheochromocytoma cell line)

whole cell lysate

Lane 9: NIH/3T3 (Mouse embryo fibroblast cell line) whole cell

lysate

Lysates/proteins at 10 µg per lane.

Secondary

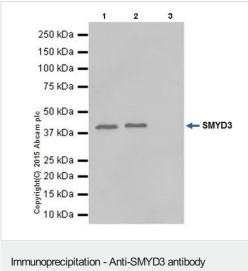
All lanes : Goat Anti-Rabbit $\lg G \ H\&L \ (HRP) \ (\underline{ab97051})$ at

1/100000 dilution

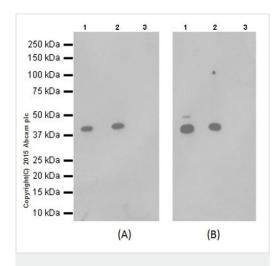
Predicted band size: 49 kDa **Observed band size:** 45 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.



Immunoprecipitation - Anti-SMYD3 antibody [EPR19311] (ab199361)



Immunoprecipitation - Anti-SMYD3 antibody [EPR19311] (ab199361)

SMYD3 was immunoprecipitated from 1mg of 293T (Human epithelial cell line from embryonic kidney) whole cell lysate with ab199361 at 1/50 dilution.

Western blot was performed from the immunoprecipitate using ab199361 at 1/1000 dilution.

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

Lane 1: 293T whole cell lysate, 10µg (Input).

Lane 2: ab199361 IP in 293T whole cell lysate.

Lane 3: Rabbit lgG,monoclonal [EPR25A]-Isotype Control (ab172730) instead of ab199361 in 293T whole cell lysate.

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

Exposure time: 3 minutes.

SMYD3 was immunoprecipitated from 1mg of 293T (Human epithelial cell line from embryonic kidney) whole cell lysate with ab199361 at 1/50 dilution.

Western blot was performed from the immunoprecipitate using ab199361 at 1/1000 dilution (Panel A) or <u>ab183498</u> at 1/1000 dilution (Panel B).

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

Lane 1: 293T whole cell lysate, 10µg (Input).

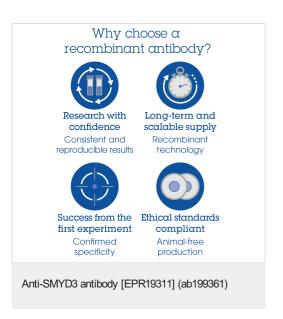
Lane 2: ab199361 IP in 293T whole cell lysate.

Lane 3: Rabbit lgG,monoclonal [EPR25A]-Isotype Control (ab172730) instead of ab199361 in 293T whole cell lysate.

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

Exposure time: 3 minutes.

The product was validated with $\underline{ab183498}$, which is specific to SMYD3.



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