abcam

Product datasheet

Anti-SESN2/Sestrin-2 antibody [EPR18907] ab178518





重组 RabMAb

11 图像 14 References

概述

产品名称 Anti-SESN2/Sestrin-2抗体[EPR18907]

描述 兔单克隆抗体[EPR18907] to SESN2/Sestrin-2

宿主 Rabbit

经测试应用 适用于: WB, ICC/IF, IP, Flow Cyt (Intra)

种属反应性 与反应: Mouse, Rat, Human

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

阳性对照 WB: HeLa whole cell lysate treated with 10 mM H2O2 for 1 hour; HeLa, LoVo, 293, NIH/3T3, HCT

> 116, Rat1, RAW 264.7, C6 and PC-12 whole cell lysates; Human colon, fetal liver, testis and fetal kidney lysates; Mouse spleen lysate. HEK-293 cell lysate. ICC/IF: HCT 116 cells. Flow Cyt (intra):

NIH/3T3 and HCT 116 cells. IP: HeLa whole cell lysate.

常规说明 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**.

性能

形式

存放说明 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.

Preservative: 0.01% Sodium azide 存储溶液

Constituents: PBS, 40% Glycerol, 0.05% BSA

纯度 Protein A purified

克隆 单克隆 克隆编号 EPR18907

同种型 IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab178518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		1/1000. Detects a band of approximately 54 kDa (predicted molecular weight: 54 kDa).
ICC/IF		1/100. ICC/IF is recommended for human and rat only.
IP		1/30.
Flow Cyt (Intra)		1/60.

靶标

图片



Western blot - Anti-SESN2/Sestrin-2 antibody [EPR18907] (ab178518)

All lanes : Anti-SESN2/Sestrin-2 antibody [EPR18907] (ab178518) at 1/1000 dilution

Lane 1: Wild-type HEK-293 cell lysate

Lane 2: SESN2 knockout HEK-293 cell lysate

Performed under reducing conditions.

Predicted band size: 54 kDa Observed band size: 54 kDa

False colour image of Western blot: Anti-SESN2/Sestrin-2 antibody [EPR18907] 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, ab178518 was shown to bind specifically to SESN2/Sestrin-2. A band was observed at 54 kDa in wild-type HEK-293 cell lysates with no signal observed at this size in SESN2 knockout cell line ab269486 (knockout cell lysate ab269650). To generate this image, wild-type

and SESN2 knockout HEK-293 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 lgG H&L (IRDye[®] 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye[®] 680RD) preabsorbed (ab216776) at 1/20000 dilution.

260 kDa-160 kDa-125 kDa-90 kDa-75 kDa-50 kDa-38 kDa-30 kDa-25 kDa-15 kDa-8 kDa-8 kDa-

Western blot - Anti-SESN2/Sestrin-2 antibody [EPR18907] (ab178518)

All lanes : Anti-SESN2/Sestrin-2 antibody [EPR18907] (ab178518) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate

Lane 2: SESN2 knockout HeLa cell lysate

Lane 3: LoVo cell lysate

Lane 4: HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

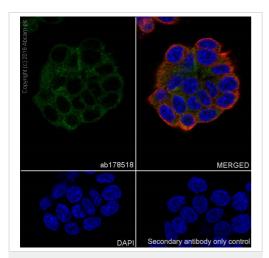
All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) at 1/10000 dilution

Predicted band size: 54 kDa **Observed band size:** 54 kDa

Lanes 1-4: Merged signal (red and green). Green - ab178518 observed at 54 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab178518 Anti-SESN2/Sestrin-2 antibody [EPR18907] was shown to specifically react with SESN2/Sestrin-2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265669 (knockout lysate ab257665) was used. Wild-type and SESN2/Sestrin-2 knockout samples were subjected to SDS-PAGE. ab178518 and Anti-GAPDH antibody [6C5] - Loading Control (ab8245) were incubated at room temperature for 2.5 hours at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in

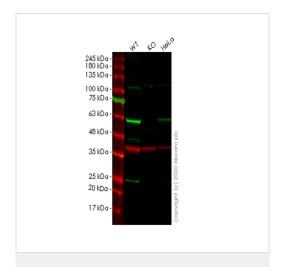
20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-SESN2/Sestrin-2 antibody [EPR18907] (ab178518)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HCT 116 (Human colorectal carcinoma cell line) cells labeling SESN2/Sestrin-2 with ab178518 at 1/100 dilution, followed by Goat anti-rabbit IgG (Alexa Fluor[®] 488) (ab150077) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on HCT116 cells. The nuclear counter stain is DAPI (blue). Tubulin is detected with ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) at 1/250 dilution (red).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat anti-rabbit lgG (Alexa Fluor[®] 488) (**ab150077**) secondary antibody at 1/1000 dilution.



Western blot - Anti-SESN2/Sestrin-2 antibody [EPR18907] (ab178518)

All lanes : Anti-SESN2/Sestrin-2 antibody [EPR18907] (ab178518) at 1/1000 dilution

Lane 1: Wild-type HeLa cell lysate

Lane 2: SESN2 knockout HeLa cell lysate

Lane 3: HeLa cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preadsorbed (ab216773) at 1/10000 dilution

Predicted band size: 54 kDa Observed band size: 54 kDa

Lanes 1-3: Merged signal (red and green). Green - ab178518 observed at 54 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

ab178518 Anti-SESN2/Sestrin-2 antibody [EPR18907] was shown to specifically react with SESN2/Sestrin-2 in wild-type HeLa cells. Loss of signal was observed when knockout cell line ab265669 (knockout cell lysate ab257665) was used. Wild-type and SESN2/Sestrin-2 knockout samples were subjected to SDS-PAGE. ab178518 and Anti-GAPDH antibody [6C5] - Loading

Control (ab8245) were incubated overnight at 4°C at 1 in 1000 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

1 2 3 4 5

250 kDa —

150 kDa —

100 kDa —

75 kDa —

50 kDa —

37 kDa —

91d u 25 kDa —

225 kDa —

226 kDa —

24 9002 15 kDa —

4 ab181602 GAPDH

Western blot - Anti-SESN2/Sestrin-2 antibody [EPR18907] (ab178518)

All lanes : Anti-SESN2/Sestrin-2 antibody [EPR18907] (ab178518) at 1/1000 dilution

Lane 1 : Untreated HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate

Lane 2: HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate treated with 10 mM H2O2 for 1 hour

Lane 3 : LoVo (Human colorectal adenocarcinoma cell line) whole cell lysate

Lane 4: 293T (Human epithelial cell line from embryonic kidney) whole cell lysate

Lane 5: NIH/3T3 (Mouse embryonic fibroblast 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

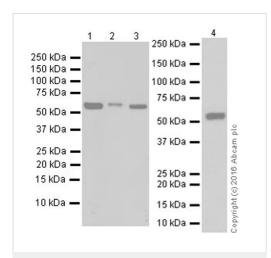
Predicted band size: 54 kDa Observed band size: 54 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

Sestrin expression is induced by $\rm H_2O_2$ treatment, which is consistent with what has been described in the literature (PMID: 25337554).

Exposure time:3 minutes



Western blot - Anti-SESN2/Sestrin-2 antibody [EPR18907] (ab178518)



(ab178518) at 1/1000 dilution

Lane 1: Human colon lysate

Lane 2: Human fetal liver lysate

Lane 3: Human testis lysate

Lane 4: Human fetal kidney lysate

Lysates/proteins at 20 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: 54 kDa Observed band size: 54 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time:3 minutes

All lanes: Anti-SESN2/Sestrin-2 antibody [EPR18907] (ab178518) at 1/1000 dilution

Lane 1: HCT 116 (Human colorectal carcinoma cell line) whole cell lysate

Lane 2: Rat1 (Rat fibroblast cell line) whole cell lysate

Lane 3: C6 (Rat glial tumor cell line) whole cell lysate

Lane 4: RAW 264.7 (Mouse macrophage cell line transformed

with Abelson murine leukemia virus) whole cell lysate

Lane 5: Mouse spleen lysate

Lane 6: PC-12 (Rat adrenal gland pheochromocytoma cell line)

whole cell lysate

Lysates/proteins at 20 µg per lane.

Western blot - Anti-SESN2/Sestrin-2 antibody [EPR18907] (ab178518)

2 3

250 kDa -150 kDa -100 kDa -

75 kDa -

50 kDa -

37 kDa -

25 kDa -20 kDa -

15 kDa -

10 kDa -

5 6

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Secondary

All lanes: Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at

Predicted band size: 54 kDa Observed band size: 54 kDa

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure times: Lane 1, 2, 3, 4 and 5: 3 minutes; Lane 6: 30

seconds.

SESN2/Sestrin-2 was immunoprecipitated from 0.35 mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab178518 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab178518 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/10000 dilution.

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

Lane 2: ab178518 IP in HeLa whole cell lysate.

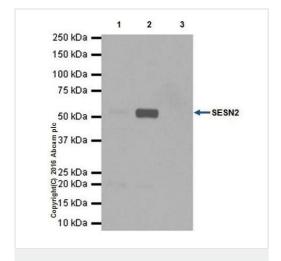
Lane 3: Rabbit lgG,monoclonal [EPR25A] - Isotype Control (ab172730) instead of ab178518 in HeLa whole cell lysate.

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

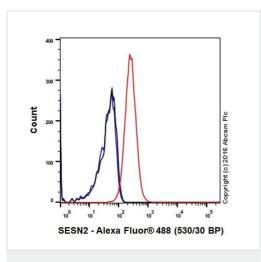
Exposure time: 3 minutes.

SESN2/Sestrin-2 expression is low in HeLa cells and can be enriched through immunoprecipitation.

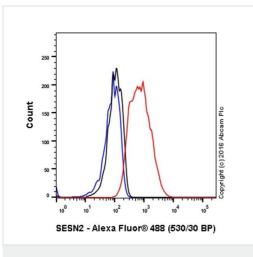
Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed NIH/3T3 (Mouse embryonic fibroblast cell line) cells labeling SESN2/Sestrin-2 with ab178518 at 1/60 dilution (red) compared with a Rabbit lgG,monoclonal[EPR25A]-Isotype control (ab172730) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit lgG (Alexa Fluor® 488) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-SESN2/Sestrin-2 antibody [EPR18907] (ab178518)



Flow Cytometry (Intracellular) - Anti-SESN2/Sestrin-2 antibody [EPR18907] (ab178518)



Flow Cytometry (Intracellular) - Anti-SESN2/Sestrin-2 antibody [EPR18907] (ab178518) Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed HCT 116 (Human colorectal carcinoma cell line) cells labeling SESN2/Sestrin-2 with ab178518 at 1/60 dilution (red) compared with a rabbit monoclonal IgG isotype control (ab172730; black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody; blue). Goat anti rabbit IgG (Alexa Fluor[®] 488) at 1/2000 dilution was used as the secondary antibody.



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