abcam

Product datasheet

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

KO VALIDATED Recombinant RabMAb

14 References 11 Images

Overview

Product name	Anti-SESN2/Sestrin-2 antibody [EPR18907]
Description	Rabbit monoclonal [EPR18907] to SESN2/Sestrin-2
Host species	Rabbit
Tested applications	**Suitable for:** WB, ICC/IF, IP, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa whole cell lysate treated with 10 mM H_2O_2 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.
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	Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18907

Isotype IgG

Applications

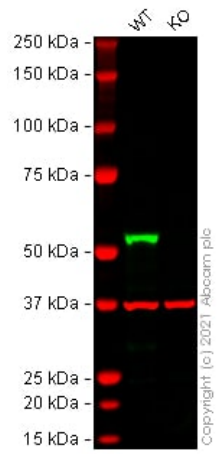
The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab178518 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		1/1000. 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.

Target

Images



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

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

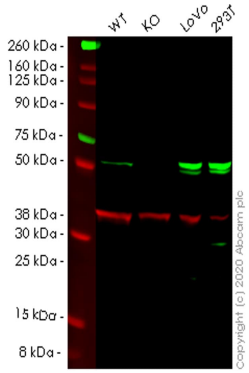
Lane 1 : Wild-type HEK-293 cell lysate
Lane 2 : SESN2 knockout HEK-293 cell lysate

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 IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 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 : LoVo cell lysate
Lane 4 : HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

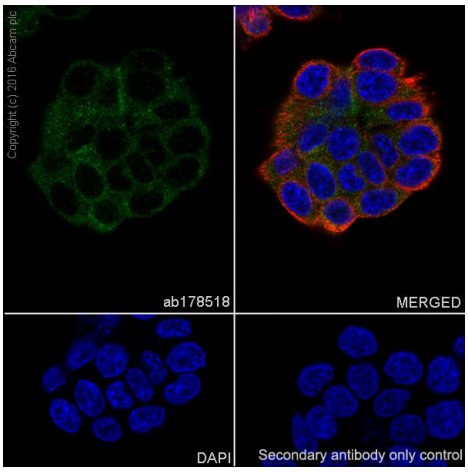
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

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 IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in

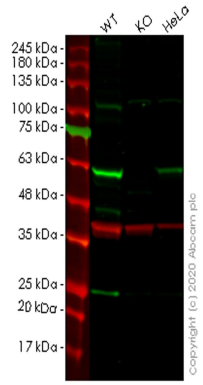
20000 dilution for 1 hour at room temperature before imaging.



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 IgG (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.

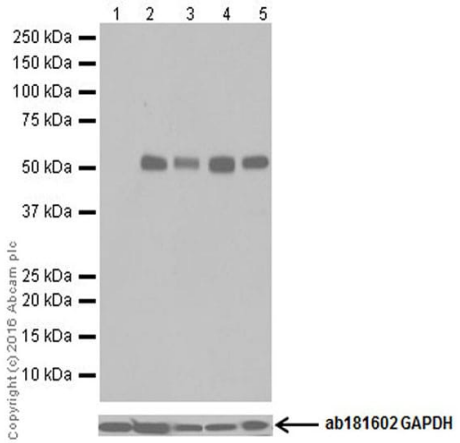
Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

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 **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 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.



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 IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

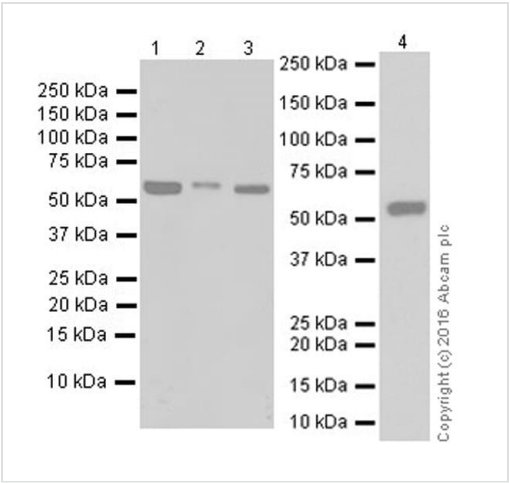
Predicted band size: 54 kDa
Observed band size: 54 kDa

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

Sestrin expression is induced by 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)

All lanes : Anti-SESN2/Sestrin-2 antibody [EPR18907] (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.

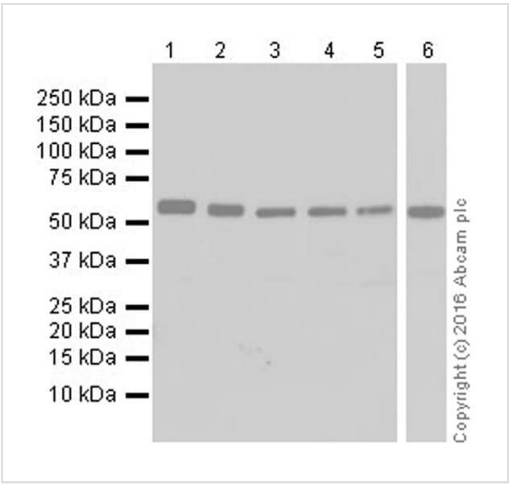
Secondary
All lanes : Goat Anti-Rabbit IgG Peroxidase Conjugate, specific to the non-reduced form of IgG at 1/10000 dilution

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

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

Exposure time:3 minutes



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

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.

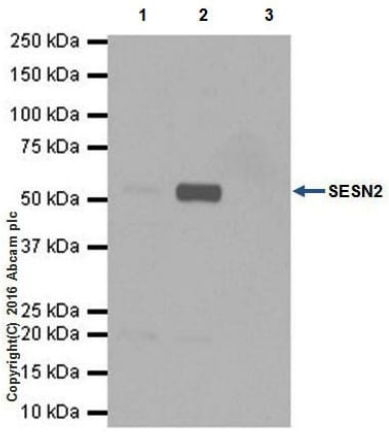
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at

1/100000 dilution

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.



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

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).

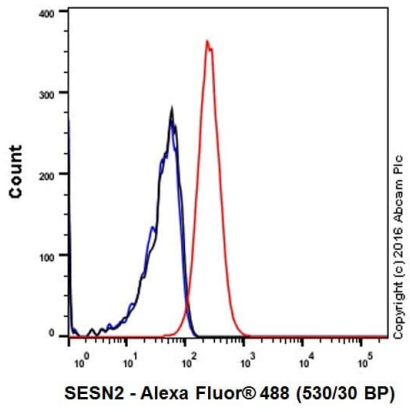
Lane 2: ab178518 IP in HeLa whole cell lysate.

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

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

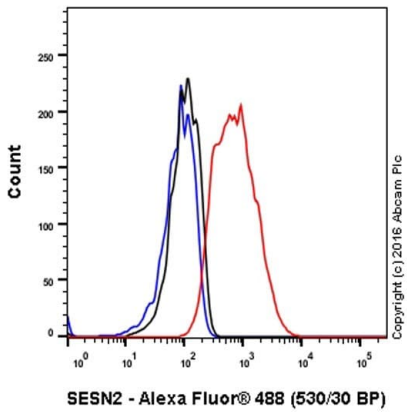
Exposure time: 3 minutes.

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



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

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 IgG,monoclonal[EPR25A]-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.



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.

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

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