abcam

ab303548

Anti-SMCR8 antibody [EPR26215-5]

Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR26215-5
Purification technique	Affinity purification Protein A
Concentration	0.526 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Species	Mouse

Dilution info	1/1000
Notes	-

Species	Rat
Dilution info	1/1000
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	1/2000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Species	Rat
Dilution info	1/2000
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Human
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

IHC-Fr

Tested

Species	Mouse
Dilution info	1/500
Notes	-

Expected

Species	Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Not recommended

Species	Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/50
Notes	-

Species	Mouse
Dilution info	1/50
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/500
Notes	-

Species	Mouse
Dilution info	1/500
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

IP

Tested

Species	Human
Dilution info	1/30
Notes	-

Species	Mouse
Dilution info	1/30
Notes	-

Expected

Species	Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information SMCR8

Function	Component of the C9orf72-SMCR8 complex, a complex that has guanine nucleotide exchange factor (GEF) activity and regulates autophagy (PubMed:20562859, PubMed:27103069, PubMed:27193190, PubMed:27559131, PubMed:27617292, PubMed:28195531, PubMed:32303654). In the complex, C9orf72 and SMCR8 probably constitute the catalytic subunits that promote the exchange of GDP to GTP, converting inactive GDP-bound RAB8A and RAB39B into

their active GTP-bound form, thereby promoting autophagosome maturation (PubMed:20562859, PubMed:27103069, PubMed:27617292, PubMed:28195531). The C9orf72-SMCR8 complex also acts as a negative regulator of autophagy initiation by interacting with the ULK1/ATG1 kinase complex and inhibiting its protein kinase activity (PubMed:27617292, PubMed:28195531). As part of the C9orf72-SMCR8 complex, stimulates RAB8A and RAB11A GTPase activity in vitro (PubMed:32303654). Acts as a regulator of mTORC1 signaling by promoting phosphorylation of mTORC1 substrates (PubMed:27559131, PubMed:28195531). In addition to its activity in the cytoplasm within the C9orf72-SMCR8 complex, SMCR8 also localizes in the nucleus, where it associates with chromatin and negatively regulates expression of suppresses ULK1 and WIPI2 genes (PubMed:28195531).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

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

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	SMCR8 also known as Smith-Magenis Syndrome Chromosome Region 8 is a protein with a molecular weight of approximately 105 kDa. This protein is expressed in multiple tissues with significant presence in the brain kidney and liver. SMCR8 functions mechanically as a guanine nucleotide exchange factor and it plays a vital role in cellular homeostasis and autophagy.
Biological function summary	SMCR8 is involved in regulating macroautophagy a critical process for cellular maintenance by forming a complex with the C9orf72 protein. This complex ensures proper lysosomal positioning and function impacting the cell's ability to remove damaged organelles and proteins. SMCR8's activity is essential for maintaining normal lysosomal dynamics and preventing the accumulation of toxic substances within the cell.
Pathways	SMCR8 participates in the autophagy and lysosome pathways. It interacts with several other proteins including UVRAG and Beclin1 which are important for autophagosome formation and maturation. SMCR8 and its associated pathways are important in coordinating proper cellular degradation and recycling processes ensuring cellular stability and function.
Associated diseases and disorders	SMCR8 has been connected to neurodegenerative conditions such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Mutations or dysfunction in SMCR8 can interfere with autophagy contributing to the pathogenesis of these disorders. The interaction between SMCR8 and C9orf72 has been particularly highlighted in relation to ALS as disruptions in this interaction could aggravate disease progression.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

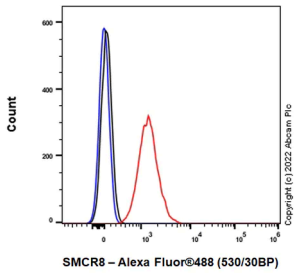
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

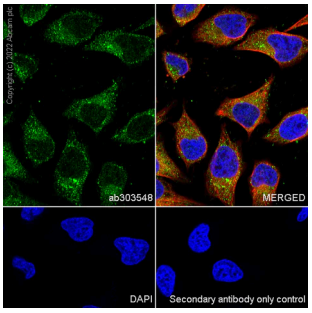
Full details and terms and conditions can be found here:
Terms & Conditions.

13 product images



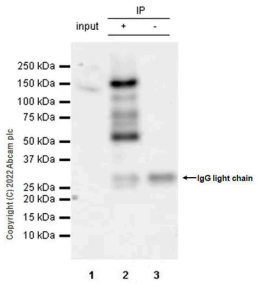
Flow Cytometry - Anti-SMCR8 antibody [EPR26215-5] (ab303548)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labelling SMCR8 with ab303548 at 1/500 dilution (0.1ug) (Red) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody.



Immunocytochemistry/ Immunofluorescence - Anti-SMCR8 antibody [EPR26215-5] (ab303548)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized HeLa (human cervix adenocarcinoma epithelial cell) cells labelling SMCR8 with ab303548 at 1/50 (10.52 ug/ml) dilution, followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 2ug/mL dilution (Green). Confocal image showing mainly cytoplasmic and weak nuclear staining in HeLa cell line is observed. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 2.5 ug/mL dilution (Red). The Nuclear counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2ug/mL dilution.



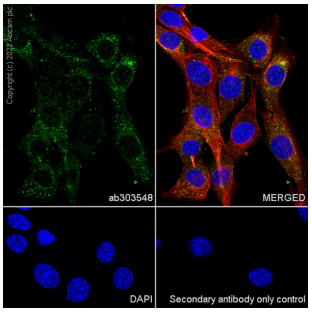
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SMCR8 was immunoprecipitated from 0.35 mg HeLa (human cervical adenocarcinoma epithelial cell), whole cell lysate 10 ug with ab303548 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab303548 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution. Lane 1: HeLa (human cervical adenocarcinoma epithelial cell), whole cell lysate 10 ug Lane 2: ab303548 IP in HeLa whole cell lysate Lane 3:Rabbit monoclonal IgG (ab172730) instead of ab303548 in HeLa whole cell lysate Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 32 seconds The bands beneath the target band in lane2 are likely to be degraded target fragments.
All lanes:
Immunoprecipitation - Anti-SMCR8 antibody [EPR26215-5] (ab303548) at 1/1000 dilution

All lanes:
HeLa (human cervical adenocarcinoma epithelial cell), whole cell lysate

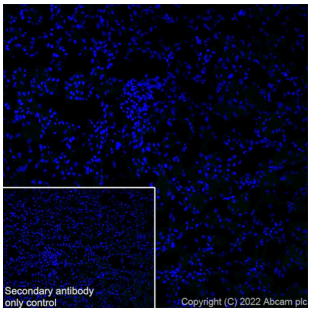
Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution



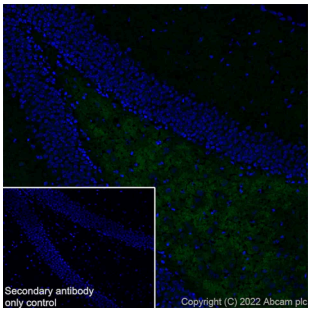
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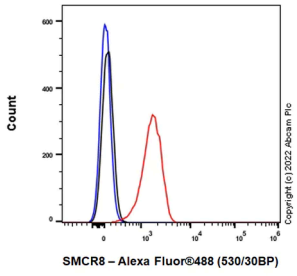
Immunohistochemistry (Frozen sections) - Anti-SMCR8 antibody [EPR26215-5] (ab303548)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Mouse kidney (fresh) tissue labeling SMCR8 with ab303548 at 1/500 (1.052 ug/ml) dilution followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/mL dilution (Green). Low expression control: confocal image showing no staining on mouse kidney (PMID: 11997338). The nuclear counterstain was DAPI (Blue). The section was incubated with ab303548 for 60mins at room temperature. The section was then mounted using Fluoromount®.The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). Secondary antibody control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbedat 1/1000 2 ug/mL dilution.



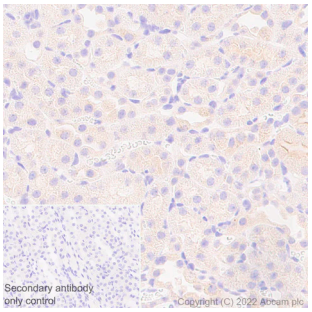
Immunohistochemistry (Frozen sections) - Anti-SMCR8 antibody [EPR26215-5] (ab303548)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Mouse hippocampus (fresh) tissue labeling SMCR8 with ab303548 at 1/500 (1.052 ug/ml) dilution followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/mL dilution (Green). Confocal image showing positive staining on mouse hippocampus. The nuclear counterstain was DAPI (Blue). The section was incubated with ab303548 for 60mins at room temperature. The section was then mounted using Fluoromount®.The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). Secondary antibody control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbedat 1/1000 2 ug/mL dilution.



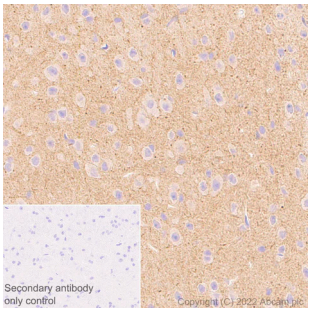
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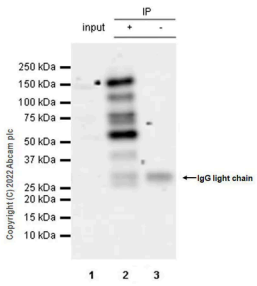
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SMCR8 antibody [EPR26215-5] (ab303548)

Immunohistochemical analysis of paraffin-embedded Mouse kidney tissue labeling SMCR8 with ab303548 at 1/2000 (0.263 ug/ml) followed by a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) was used. Weakly cytoplasmic staining on mouse kidney (PMID: 27103069). The section was incubated with ab303548 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection).Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-SMCR8 antibody [EPR26215-5] (ab303548)

Immunohistochemical analysis of paraffin-embedded Mouse cerebrum tissue labeling SMCR8 with ab303548 at 1/2000 (0.263 ug/ml) followed by a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection) was used. Positive staining on mouse cerebrum (PMID: 27103069). The section was incubated with ab303548 for 30 mins at room temperature.The immunostaining was performed on a Leica Biosystems BOND® RX instrument Counterstained with Hematoxylin. Secondary antibody only control: Secondary antibody is a ready to use LeicaDS9800 (Bond☒ Polymer Refine Detection).Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



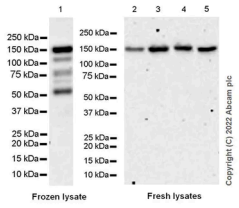
Immunoprecipitation - Anti-SMCR8 antibody [EPR26215-5] (ab303548)

SMCR8 was immunoprecipitated from 0.35 mg NIH/3T3 (mouse embryonic fibroblast), whole cell lysate 10 ug with ab303548 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab303548 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(ab131366) was used at 1/5000 dilution. Lane 1: NIH/3T3 (mouse embryonic fibroblast), whole cell lysate 10 ug Lane 2: ab303548 IP in NIH/3T3 whole cell lysate Lane 3:Rabbit monoclonal IgG (ab172730) instead of ab303548 in NIH/3T3 whole cell lysate Blocking and dilution buffer and concentration: 5% NFDM/TBST. Exposure time: 32 seconds The bands beneath the target band in lane2 are likely to be degraded target fragments.
All lanes:
NIH/3T3 (mouse embryonic fibroblast), whole cell lysate

Secondary

All lanes:
Immunoprecipitation - VeriBlot for IP Detection Reagent (HRP) (ab131366) at 1/5000 dilution

Exposure time: 32s



Western blot - Anti-SMCR8 antibody [EPR26215-5] (ab303548)

SMCR8 Western blot staining using rabbit Anti-SMCR8 antibody

Blocking and dilution buffer and concentration: 5% NFDM/TBST. The molecular weight observed is consistent with what has been described in the literature (PMID: 27103069). The bands beneath the target band are likely to be degraded target fragments. In lanes 1, the lysate was stored at -80☒ prior to Western Blotting. In lane 2-5, the lysates were freshly made and used for Western

Blotting immediately to minimize protein degradation. Exposure time: Lane 1: 84 seconds Lane 2-5: 3 minutes
All lanes:
Western blot - Anti-SMCR8 antibody [EPR26215-5] (ab303548) at 1/1000 dilution

Lanes 1 - 2:
HeLa (human cervical adenocarcinoma epithelial cell) at 20 µg

Lane 3:
HEK 293T (human embryonic kidney epithelial cell) at 20 µg

Lane 4:
NIH/3T3 (mouse embryonic fibroblast), whole cell fresh lysate at 20 µg

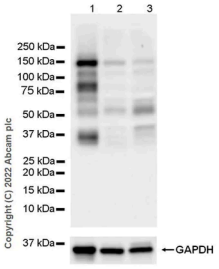
Lane 5:
PC-12 (rat adrenal gland pheochromocytoma), whole cell fresh lysate at 20 µg

Secondary

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

Predicted band size: 105 kDa

Observed band size: 140 kDa



Western blot - Anti-SMCR8 antibody [EPR26215-5] (ab303548)

SMCR8 Western blot staining using rabbit Anti-SMCR8 antibody

Blocking and dilution buffer and concentration: 5% NFDM/TBST. The molecular weight observed is consistent with what has been described in the literature (PMID: 27103069). The bands beneath the target band are likely to be degraded target fragments. Low expression: kidney, lung (PMID: 27103069). Exposure time Lane 1-4: 81 seconds Lane 5-7: 3 minutes.
All lanes:
Western blot - Anti-SMCR8 antibody [EPR26215-5] (ab303548) at 1/1000 dilution

Lane 1:
Human cerebellum tissue lysate at 20 µg

Lane 2:
Human kidney tissue lysate at 20 µg

Lane 3:
Human lung tissue lysate at 20 µg

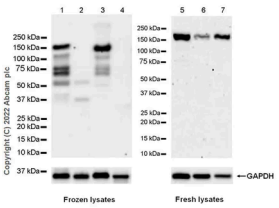
Secondary

All lanes:
Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 105 kDa

Observed band size: 140 kDa

Western blot - Anti-SMCR8 antibody [EPR26215-5] (ab303548)



SMCR8 Western blot staining using rabbit Anti-SMCR8 antibody

Blocking and dilution buffer and concentration: 5% NFDM/TBST. The molecular weight observed is consistent with what has been described in the literature (PMID: 27103069). The bands beneath the target band are likely to be degraded target fragments. Low expression: kidney (PMID: 27103069) In lanes 1-4, the lysates were stored at -80☒ prior to Western Blotting. In lane 5-7, the lysates were freshly made and used for Western Blotting immediately to minimize protein degradation. Exposure time: Lane 1-4: 81 seconds Lane 5-7: 3 minutes
All lanes:
Western blot - Anti-SMCR8 antibody [EPR26215-5] (ab303548) at 1/1000 dilution

Lane 1:
Mouse cerebral cortex tissue lysate at 20 µg

Lane 2:
Mouse kidney tissue lysate at 20 µg

Lane 3:
Rat cerebral cortex tissue lysate at 20 µg

Lane 4:
Rat kidney tissue lysate at 20 µg

Lane 5:
Mouse brain fresh tissue lysate at 20 µg

Lane 6:
Mouse liver fresh tissue lysate at 20 µg

Lane 7:
Mouse testis fresh tissue lysate at 20 µg

Secondary

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

Predicted band size: 105 kDa

Observed band size: 140 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.