abcam

Product datasheet

Anti-SCA2 antibody [EPR23630-49] ab254362

KO VALIDATED Recombinant RabMAb

13 Images

Overview

Product name	Anti-SCA2 antibody [EPR23630-49]
Description	Rabbit monoclonal [EPR23630-49] to SCA2
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), WB, IHC-P, IHC-Fr, ICC/IF Unsuitable for: IP
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, HEK-293T, NIH/3T3 and PC-12 whole cell lysate. IHC-P: Human cerebrum tissue. Human, mouse and rat cerebellum tissue. IHC-Fr: Mouse cerebrum and cerebellum tissue. ICC/IF: HeLa and NIH/3T3 cells. Flow Cyt (intra): HeLa and NIH/3T3 cells.
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 <u>see here</u>. Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb[®] patents</u>.

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: 59.94% PBS, 0.05% BSA, 40% Glycerol
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR23630-49

Applications

The Abpromise guaranteeOur Abpromise guaranteecovers the use of ab254362 in the following tested applications.The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

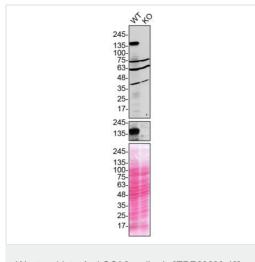
Application	Abreviews	Notes
Flow Cyt (Intra)		1/500.
WB		1/1000. Detects a band of approximately 41, 145 kDa (predicted molecular weight: 140 kDa).
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
IHC-Fr		1/50. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).
ICC/IF		1/50.

Application notes

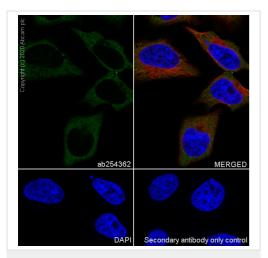
Is unsuitable for IP.

Target	
Function	Involved in EGFR trafficking, acting as negative regulator of endocytic EGFR internalization at the plasma membrane.
Tissue specificity	Expressed in the brain, heart, liver, skeletal muscle, pancreas and placenta. Isoform 1 is predominant in the brain and spinal cord. Isoform 4 is more abundant in the cerebellum. In the brain, broadly expressed in the amygdala, caudate nucleus, corpus callosum, hippocampus, hypothalamus, substantia nigra, subthalamic nucleus and thalamus.
Involvement in disease	Spinocerebellar ataxia 2 Amyotrophic lateral sclerosis 13
Sequence similarities	Belongs to the ataxin-2 family.
Cellular localization	Cytoplasm.

Images



Western blot - Anti-SCA2 antibody [EPR23630-49] (ab254362) ab254362 was shown to react with aTXN2 in wild-type HAP1 cells in Western blot with loss of signal observed in a ATXN2 knockout cell line. Wild-type HAP1 and ATXN2 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 5% milk in TBST for 1 hr before incubation with ab254362 overnight at 4 °C at a 1/1000 dilution. Blots were incubated with goat anti-rabbit HRP secondary antibodies at before imaging. These data were provided by YCharOS Inc., an open science company with the mission of characterizing commercially available antibody reagents for all human proteins. Abcam and YCharOS are working together to help address the reproducibility crisis by enabling the life science community to better evaluate commercially available antibodies.



Immunocytochemistry/ Immunofluorescence - Anti-SCA2 antibody [EPR23630-49] (ab254362) Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling SCA2 with ab254362 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (<u>ab150077</u>) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on HeLa cell line. The nuclear counterstain is DAPI (blue).

Tubulin is detected with <u>ab195889</u> Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor[®] 594) at 1/200 dilution (red).

The negative control is secondary antibody only.



Western blot - Anti-SCA2 antibody [EPR23630-49] (ab254362) All lanes : Anti-SCA2 antibody [EPR23630-49] (ab254362) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lane 2 : HEK-293T (human epithelial cell line from embryonic kidney transformed with large T antigen) whole cell lysate Lane 3 : NIH/3T3 (mouse embryonic fibroblast) whole cell lysate Lane 4 : PC-12 (rat adrenal gland pheochromocytoma) 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

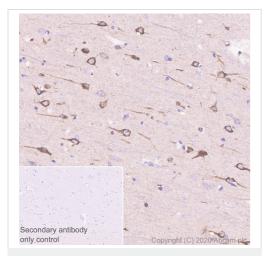
Predicted band size: 140 kDa Observed band size: 145,41 kDa

The molecular weight observed is consistent with what has been described in the literature (PMID: 9989626).

Lysates should be made freshly and used in WB immediately to minimize protein degradation.

Blocking/Dilution buffer: 5% NFDM/TBST.

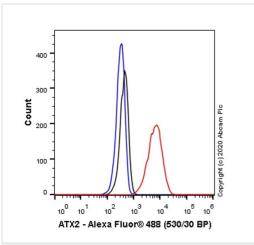
Exposure times: Lanes 1-3: 10 seconds; Lane 4: 15 seconds.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SCA2 antibody [EPR23630-49] (ab254362)

Immunohistochemical analysis of paraffin-embedded human cerebrum tissue labeling SCA2 with ab254362 at 1/1000 dilution, followed by a ready to use secondary from Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>). Cytoplasmic staining on neurons of human cerebrum (PMID: 9989626). The section was incubated with ab254362 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND[®] RX instrument. Counter stained with Hematoxylin.

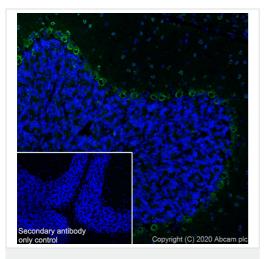
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use secondary from Rabbit specific IHC polymer detection kit HRP/DAB (<u>ab209101</u>).



Flow Cytometry (Intracellular) - Anti-SCA2 antibody [EPR23630-49] (ab254362) Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling SCA2 with ab254362 at 1/500 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 H&L (Alexa Fluor[®] 488) at 1/2000 dilution was used as the secondary antibody.

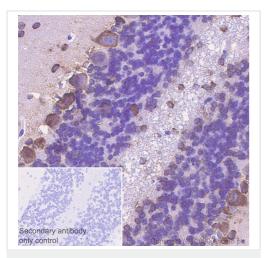


Immunohistochemistry (Frozen sections) - Anti-SCA2 antibody [EPR23630-49] (ab254362)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen mouse cerebellum tissue labeling SCA2 with ab254362 at 1/50 dilution, followed by <u>ab150077</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) at a 1/1000 dilution. Positive staining on mouse cerebellum is observed. Nuclear counterstain is DAPI. Secondary antibody only control: Used PBS instead of primary

antibody, secondary antibody only control. Cosed 1 Do Instead of primary antibody, secondary antibody is <u>**ab150077**</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) at a 1/1000 dilution.

Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).

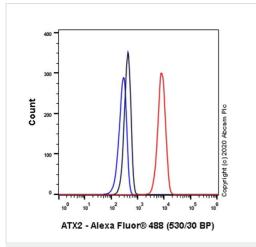


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SCA2 antibody [EPR23630-49] (ab254362)

Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue labeling SCA2 with ab254362 at 1/1000 dilution, followed by a ready to use secondary from Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Cytoplasmic staining in rat cerebellum (PMID: 26868665). The section was incubated with ab254362 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND[®] RX instrument. Counter stained with Hematoxylin.

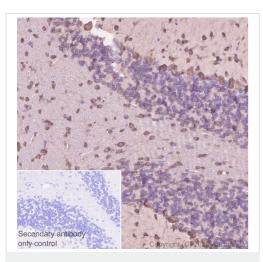
Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use secondary from Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol permeabilized NIH/3T3 (mouse embryo fibroblast cell line) cells labeling SCA2 with ab254362 at 1/500 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 H&L (Alexa Fluor[®] 488) at 1/2000 dilution was used as the secondary antibody.

Flow Cytometry (Intracellular) - Anti-SCA2 antibody [EPR23630-49] (ab254362)

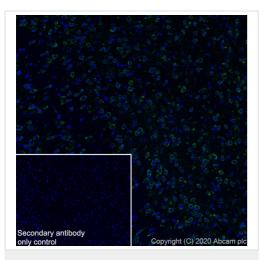


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SCA2 antibody [EPR23630-49] (ab254362)

Immunohistochemical analysis of paraffin-embedded mouse cerebellum tissue labeling SCA2 with ab254362 at 1/1000 dilution, followed by a ready to use secondary from Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Cytoplasmic staining in mouse cerebellum (PMID: 26868665). The section was incubated with ab254362 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND[®] RX instrument. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use secondary from Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).

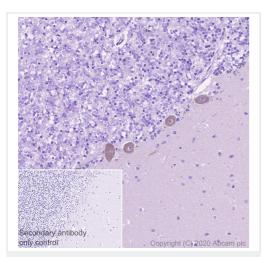
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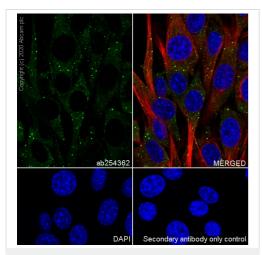
Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-SCA2 antibody [EPR23630-49] (ab254362) Immunohistochemical analysis of paraffin-embedded human cerebellum tissue labeling SCA2 with ab254362 at 1/1000 dilution, followed by a ready to use secondary from Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Cytoplasmic staining on Purkinje cells in human cerebellum (PMID: 9989626). The section was incubated with ab254362 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND[®] RX instrument. Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use secondary from Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0,

epitope retrieval solution2) for 20 mins.



Immunocytochemistry/ Immunofluorescence - Anti-SCA2 antibody [EPR23630-49] (ab254362)



Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized NIH/3T3 (mouse embryo fibroblast cell line) cells labeling SCA2 with ab254362 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor[®] 488) (**ab150077**) secondary antibody at 1/1000 dilution (green). Confocal image showing cytoplasmic staining on NIH/3T3 cell line. The nuclear counterstain is DAPI (blue).

Tubulin is detected with **ab195889** Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor[®] 594) at 1/200 dilution (red).

The negative control is secondary antibody only.

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