abcam

Product datasheet

Anti-TMS1/ASC antibody [EPR23978-28] ab283684

KO VALIDATED Recombinant RabMAb

3 References 9 Images

Overview

Product name	Anti-TMS1/ASC antibody [EPR23978-28]	
Description	Rabbit monoclonal [EPR23978-28] to TMS1/ASC	
Host species	Rabbit	
Tested applications	Suitable for: Flow Cyt (Intra), ICC/IF, WB, IP, IHC-P Unsuitable for: IHC-Fr	
Species reactivity	Reacts with: Human	
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.	
Positive control	WB: Human spleen, colon and tonsil tissue lysate; Wild-type HAP1 and THP-1 cell lysates. IHC-P: Human tonsil, colon and colon cancer tissue. ICC/IF: THP-1 cells. Flow Cyt: HeLa cells. IP: THP-1 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. 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	EPR23978-28

Applications

The Abpromise guaranteeOur Abpromise guaranteecovers the use of ab283684 in the following tested applications.

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

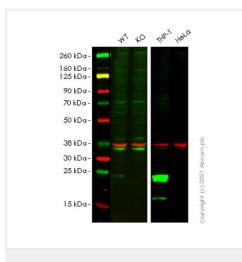
Application	Abreviews	Notes
Flow Cyt (Intra)		1/5000.
ICC/IF		1/100.
WB		1/1000. Predicted molecular weight: 22 kDa.
IP		1/30.
IHC-P		1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Application notes

Is unsuitable for IHC-Fr.

Target	
Function	Promotes caspase-mediated apoptosis. This proapoptotic activity is mediated predominantly through the activation of caspase-9. May be a component of the inflammasome, a protein complex which also includes NALP2, CARD8 and CASP1 and whose function would be the activation of proinflammatory caspases.
Tissue specificity	Widely expressed at low levels. Detected in peripheral blood leukocytes, lung, small intestine, spleen, thymus, colon and at lower levels in placenta, liver and kidney. Very low expression in skeletal muscle, heart and brain. Detected in the leukemia cell lines HL-60 and U937, but not in Jurkat T-cell lymphoma and Daudi Burkitt's lymphoma. Detected in the melanoma cell line WM35, but not in WM793. Not detected in HeLa cervical carcinoma cells and Molt 4 lymphocytic leukemia cells.
Sequence similarities	Contains 1 CARD domain. Contains 1 DAPIN domain.
Domain	Interacts with CIAS1/PYPAF1 and PYDC1 via the DAPIN domain.
Post-translational modifications	Phosphorylated.
Cellular localization	Cytoplasm. Upstream of caspase activation, a redistribution from the cytoplasm to the aggregates occurs. These appear as hollow, perinuclear spherical, ball-like structures.

Images



Western blot - Anti-TMS1/ASC antibody [EPR23978-28] (ab283684)

All lanes : Anti-TMS1/ASC antibody [EPR23978-28] (ab283684) at 1/1000 dilution

Lane 1 : Wild-type HAP1 cell lysate

Lane 2 : TMS1/ASC knockout HAP1 cell lysate

Lane 3 : THP-1 (human monocytic leukemia monocyte) whole cell lysate

Lane 4 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (IRDye® 800CW) (<u>ab216773</u>) and Goat Anti-Mouse lgG H&L (IRDye® 680RD) (<u>ab216776</u>) at 1/10000 dilution

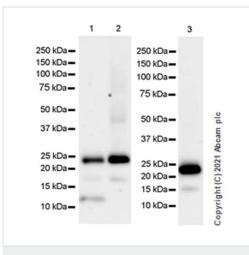
Predicted band size: 22 kDa Observed band size: 17,24 kDa

Blocking and diluting buffer and concentration: 3% NFDM/TBST

Negative control: HeLa (PMID:11103776;10567338).

Lanes 1-4: Merged signal (red and green). Green - ab283684 observed at 24 kDa. Red - loading control <u>ab8245</u> observed at 36 kDa.

Ab283684 Anti-TMS1/ASC antibody [EPR23978-28] was shown to specifically react with TMS1/ASC in wild-type HAP1 cells. Loss of signal was observed when knockout cell line (knockout cell lysate) was used. Wild-type and TMS1/ASC knockout samples were subjected to SDS-PAGE. ab283684 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.



Western blot - Anti-TMS1/ASC antibody [EPR23978-28] (ab283684) All lanes : Anti-TMS1/ASC antibody [EPR23978-28] (ab283684) at 1/1000 dilution

Lane 1 : Human spleen tissue lysate Lane 2 : Human colon tissue lysate Lane 3 : Human tonsil tissue lysate

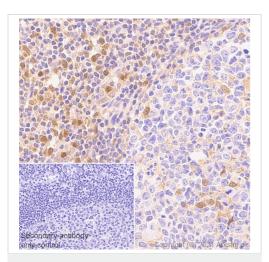
Lysates/proteins at 20 µg per lane.

Secondary

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

Predicted band size: 22 kDa Observed band size: 12,17,24 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST ASC has four isoforms. 24-kDa ASC, 17-kDa ASC-b and 12-kDa ASC-C are observed. The molecular weight observed is consistent with what has been described in the literature (PMID:20482797). Exposure time: 81 seconds

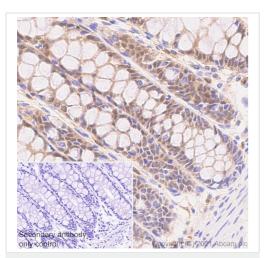


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TMS1/ASC antibody [EPR23978-28] (ab283684)

Immunohistochemical analysis of paraffin-embedded Human tonsil tissue labelling TMS1/ASC with ab283684 at 1/2000 (0.247 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond™ Polymer Refine Detection). Positive staining on human tonsil. The section was incubated with ab283684 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).

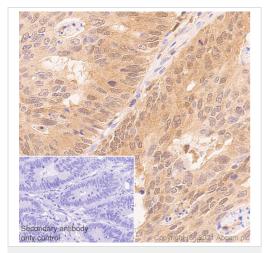
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TMS1/ASC antibody [EPR23978-28] (ab283684)

Immunohistochemical analysis of paraffin-embedded Human colon tissue labelling TMS1/ASC with ab283684 at 1/2000 (0.247 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond [™] Polymer Refine Detection). Positive staining on human colon. The section was incubated with ab283684 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) was used. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins

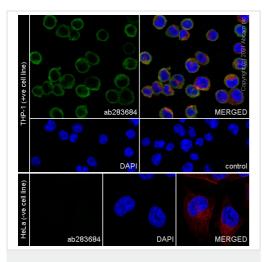


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-TMS1/ASC antibody [EPR23978-28] (ab283684)

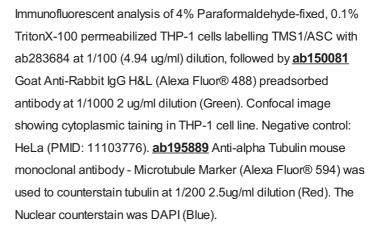
Immunohistochemical analysis of paraffin-embedded Human colon cancer tissue labelling TMS1/ASC with ab283684 at 1/2000 (0.247 ug/ml) dilution followed by a ready to use LeicaDS9800 (Bond[™] Polymer Refine Detection). Positive staining on human colon cancer (PMID: 14643031). The section was incubated with ab283684 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).

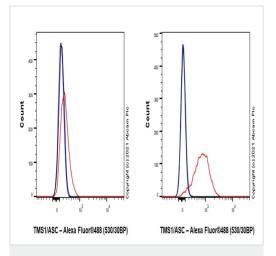
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins



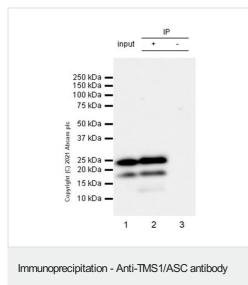
Immunocytochemistry/ Immunofluorescence - Anti-TMS1/ASC antibody [EPR23978-28] (ab283684)



Secondary antibody only control: Secondary antibody is <u>ab150081</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 2 ug/ml dilution.



Flow Cytometry (Intracellular) - Anti-TMS1/ASC antibody [EPR23978-28] (ab283684) Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized HeLa (human cervix adenocarcinoma epithelial cell, Left) / THP-1 (Human monocytic leukemia monocyte, Right) cells labelling TMS1/ASC with ab283684 at 1/5000 dilution (0.01ug) (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. Negative control: HeLa (PMID: 11103776).



[EPR23978-28] (ab283684)

TMS1/ASC was immunoprecipitated from 0.35 mg THP-1 (human monocytic leukemia monocyte) whole cell lysate 20 ug with ab283684 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab283684 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (**ab131366**) was used at 1/5000 dilution.

Lane 1: THP-1 (human monocytic leukemia monocyte) whole cell lysate 20 ug

Lane 2: ab283684 IP in THP-1 whole cell lysate

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab283684 in THP-1 whole cell lysate

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

Exposure time: 10 seconds



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