

# Product datasheet

# Anti-MUPP1 antibody [EPR26317-59] ab302621

Recombinant RabMAb

# 13 Images

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Overview			
Product name	Anti-MUPP1 antibody [EPR26317-59]		
Description	Rabbit monoclonal [EPR26317-59] to Mupp1		
Host species	Rabbit		
Specificity	IHC application not suitable with human samples.		
	ICC/IF application not suitable with human and rat samples.		
	IP application not suitable with mouse samples.		
Tested applications	Suitable for: IP, Flow Cyt (Intra), IHC-P, WB, IHC-Fr, ICC/IF		
Species reactivity	Reacts with: Mouse, Rat, Human		
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.		
Positive control	WB: C6, Neuro-2a, U-87 MG, SH-SY5Y, PC-12, mlMCD3, whole cell lysates; human cerebellun mouse brain, skeletal muscle, and testis tissue lysates; rat brain and skeletal muscle tissue lysates. IHC-P: mouse and rat choroid plexus FFPE tissue sections. IHC-Fr: mouse and rat choroid plexus fresh frozen tissue sections. ICC/IF: mlMCD3 cell line. Flow Cyt (Intra): A549 and mlMCD3 cells. IP: C6 whole cell lysate.		
General notes	This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact <b>orders@abcam.com</b> .		
	<ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <li>High batch-to-batch consistency and reproducibility</li> <li>Improved sensitivity and specificity</li> <li>Long-term security of supply</li> <li>Animal-free production</li> <li>For more information <u>see here</u>.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u>.</li> </ul>		

# Properties

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	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR26317-59
lsotype	lgG

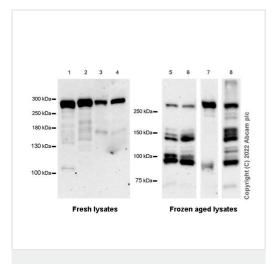
# Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab302621 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
IP		1/30.
Flow Cyt (Intra)		1/50.
IHC-P		1/800. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		1/1000. Detects a band of approximately 280 kDa (predicted molecular weight: 219 kDa).
IHC-Fr		1/50.
ICC/IF		1/50.

Target	
Relevance	MUPP1 was first identified as a protein interacting with type 2C serotonin receptor. It acts as a scaffolding protein at tight junctions where it has been reported to interact with integral proteins, anchoring them to the F-actin cytoskeleten. It is also thought to be important in the osmotic stress response in kidney cells and has been shown to play a role in promoting G protein coupling to receptors.
Cellular localization	Plasma membrane
Images	



Western blot - Anti-MUPP1 antibody [EPR26317-59] (AB302621)

All lanes : Anti-MUPP1 antibody [EPR26317-59] (ab302621) at 1/1000 dilution

Lane 1 : C6 (rat glial tumor glial cell), whole cell fresh lysate at 20 µg

Lane 2 : Neuro-2a (mouse neuroblastoma neuroblast), whole cell fresh lysate at 20 µg

Lane 3 : U-87 MG (human glioblastoma-astrocytoma epithelial cell), whole cell fresh lysate at 20 µg

Lane 4 : SH-SY5Y (human neuroblastoma epithelial cell), whole cell fresh lysate at 20 µg

Lane 5 : C6 (rat glial tumor glial cell), whole cell lysate at 40  $\mu$ g Lane 6 : PC-12 (rat adrenal gland pheochromocytoma), whole cell lysate at 40  $\mu$ g

Lane 7 : human cerebellum tissue lysate at 40  $\mu$ g

Lane 8 : mIMCD3 (mouse inner medullary collecting duct epithelial cell), whole cell lysate at 40  $\mu g$ 

# Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 219 kDa Observed band size: 280 kDa

Blocking / Diluting buffer and concentration: 5% NFDM/TBST

Exposure time:

Lane 1-7: 3 minutes

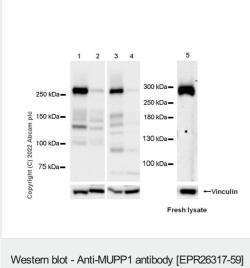
Lane 8: 26 seconds

The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 12403818).

Lysates in lane1-4 were freshly made and used for Western blotting immediately to minimize protein degradation.

The bands beneath the target band are likely to be degraded target fragments.

Samples are non-boiled as boiling may cause protein aggregates.



Western blot - Anti-MUPP1 antibody [EPR26317-59] (AB302621) All lanes : Anti-MUPP1 antibody [EPR26317-59] (ab302621) at 1/1000 dilution

- Lane 1 : Mouse brain tissue lysate at 40  $\mu g$
- Lane 2 : Mouse skeletal muscle tissue lysate at 40  $\mu g$
- Lane 3 : Rat brain tissue lysate at 40 µg
- Lane 4 : Rat skeletal muscle tissue lysate at 40  $\mu g$
- Lane 5 : Mouse testis tissue lysate at 20  $\mu$ g

### Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 219 kDa Observed band size: 280 kDa

Blocking / Diluting buffer and concentration: 5% NFDM/TBST

Exposure time:

Lane 1-4: 26 seconds

Lane 5: 3 minutes

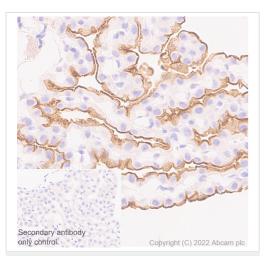
The expression profile/ molecular weight observed is consistent with what has been described in the literature (PMID: 12403818).

Low expression: skeletal muscle (PMID: 12706259)

Lysate in lane 5 was freshly made and used for Western blotting immediately to minimize protein degradation.

The bands beneath the target band are likely to be degraded target fragments.

Samples are non-boiled as boiling may cause protein aggregates.



Immunohistochemistry (Formalin/PFA-fixed paraffin-

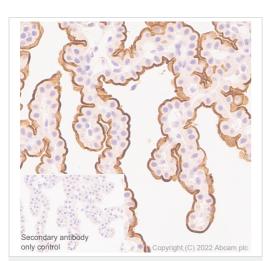
embedded sections) - Anti-MUPP1 antibody

[EPR26317-59] (AB302621)

choroid plexus tissue labeling MUPP1 with ab302621 at 1/800 (0.683 µg/ml) followed by a ready to use Leica DS9800 (Bond™ Polymer Refine Detection kit). Positive staining in mouse choroid plexus (PMID:30518636, PMID:12706259). The section was incubated with ab302621 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument. Counterstained with Hematoxylin.

Immunohistochemical analysis of paraffin-embedded mouse

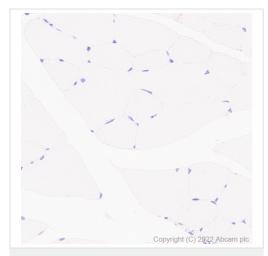
Secondary antibody only control: Secondary antibody is a ready to use Leica DS9800 (Bond<sup>™</sup> Polymer Refine Detection kit). Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MUPP1 antibody [EPR26317-59] (AB302621) Immunohistochemical analysis of paraffin-embedded rat choroid plexus tissue labeling MUPP1 with ab302621 at 1/800 (0.683 µg/ml) followed by a ready to use Leica DS9800 (Bond™ Polymer Refine Detection kit). Positive staining in rat choroid plexus (PMID:30518636, PMID:12706259 ). The section was incubated with ab302621 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Leica DS9800 (Bond™ Polymer Refine Detection kit).

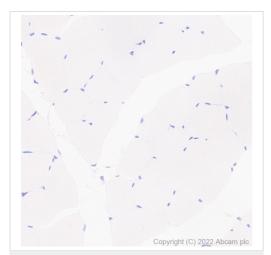
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MUPP1 antibody [EPR26317-59] (AB302621) Immunohistochemical analysis of paraffin-embedded mouse skeletal muscle tissue labeling MUPP1 with ab302621 at 1/800 (0.683 µg/ml) followed by a ready to use Leica DS9800 (Bond<sup>™</sup> Polymer Refine Detection kit). Negative control: no staining in mouse skeletal muscle (PMID:12706259). The section was incubated with ab302621 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Leica DS9800 (Bond™ Polymer Refine Detection kit).

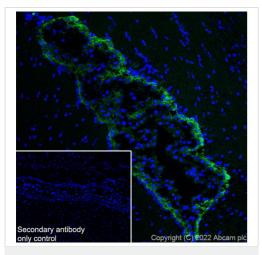
Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-MUPP1 antibody [EPR26317-59] (AB302621) Immunohistochemical analysis of paraffin-embedded rat skeletal muscle tissue labeling MUPP1 with ab302621 at 1/800 (0.683 µg/ml) followed by a ready to use Leica DS9800 (Bond™ Polymer Refine Detection kit). Negative control: no staining in rat skeletal muscle (PMID:12706259). The section was incubated with ab302621 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND<sup>®</sup> RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Leica DS9800 (Bond™ Polymer Refine Detection kit).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 mins

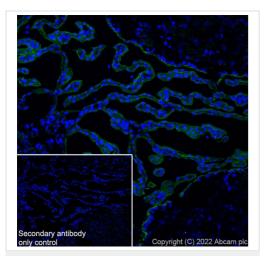


Immunohistochemistry (Frozen sections) - Anti-MUPP1 antibody [EPR26317-59] (AB302621)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen mouse choroid plexus tissue labeling MUPP1 with ab302621 at 1/50 (10.92 µg/ml) dilution followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed at 1/1000 (2 µg/ml) dilution (Green). Positive staining on mouse choroid plexus is observed. The nuclear counterstain was DAPI (Blue).

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

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

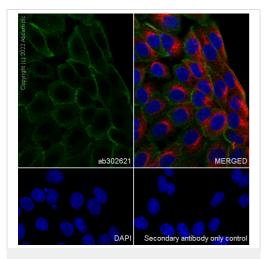


Immunohistochemistry (Frozen sections) - Anti-MUPP1 antibody [EPR26317-59] (AB302621)

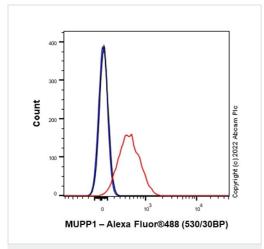
Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen rat choroid plexus tissue labeling MUPP1 with ab302621 at 1/50 (10.92 µg/ml) dilution followed by **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed at 1/1000 (2 µg/ml) dilution (Green). Positive staining on rat choroid plexus is observed. The nuclear counterstain was DAPI (Blue). Secondary antibody control: Secondary antibody is **ab150081** Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed at 1/1000 (2

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

µg/ml) dilution.<\p>



Immunocytochemistry/ Immunofluorescence - Anti-MUPP1 antibody [EPR26317-59] (AB302621)

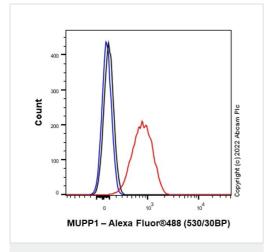


Flow Cytometry (Intracellular) - Anti-MUPP1 antibody [EPR26317-59] (ab302621)

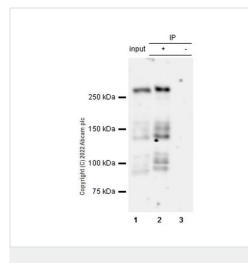
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized mIMCD3 (mouse inner medullary collecting duct epithelial cell) cells labeling MUPP1 with AB302621 at 1/50 (10.92 µg/ml) dilution, followed by <u>ab150081</u> Goat Anti-Rabbit IgG H&L (Alexa Fluor<sup>®</sup> 488) preadsorbed antibody at 1/1000 (2 µg/ml) dilution (Green). Confocal image showing membranous and cytoplasmic staining in mIMCD3 cell line is observed. <u>ab195889</u> Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor<sup>®</sup> 594) was used to counterstain tubulin at 1/200 (2.5µg/ml) dilution (Red). The Nuclear counterstain was DAPI (Blue).

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

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized mIMCD3 (mouse inner medullary collecting duct epithelial cell) cells labeling MUPP1 with ab302621 at 1/500 dilution (0.1µg) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor<sup>®</sup> 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody.



Flow Cytometry (Intracellular) - Anti-MUPP1 antibody [EPR26317-59] (ab302621)



Immunoprecipitation - Anti-MUPP1 antibody [EPR26317-59] (AB302621) Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized A549 (human lung carcinoma epithelial cell) cells labeling MUPP1 with ab302621 at 1/500 dilution (0.1µg) (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor<sup>®</sup> 488, **ab150081**) at 1/2000 dilution was used as the secondary antibody.

MUPP1 was immunoprecipitated from 0.35 mg C6 (rat glial tumor glial cell), whole cell lysate 10 µg with ab302621 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab302621 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(**ab131366**) was used at 1/5000 dilution.

Lane 1: C6 (rat glial tumor glial cell), whole cell lysate 10 µg

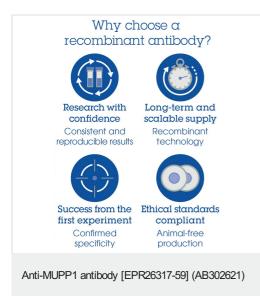
Lane 2: ab302621 IP in C6 whole cell lysate

Lane 3:Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab302621 in C6 whole cell lysate

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

Exposure time: 58 seconds

This blot was developed using a high sensitivity ECL substrate. The bands beneath the target band are likely to be degraded target fragments.



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