abcam

Product datasheet

Anti-Myelin PLP antibody [EPR23504-106] ab254363

Recombinant RabMAb

1 References 17 Images

Overview

Product name Anti-Myelin PLP antibody [EPR23504-106]

Description Rabbit monoclonal [EPR23504-106] to Myelin PLP

Host species Rabbit

Suitable for: ICC/IF, IHC-P, IP, WB, mIHC, IHC-Fr **Tested applications**

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Human brain tissue lysate; Mouse brain tissue lysate; Rat brain tissue lysate. IHC-P: Mouse

> cerebrum tissue; Human cerebrum tissue; Rat cerebrum tissue. IHC-Fr: Mouse cerebrum and cerebellum tissue; Rat cerebrum and cerebellum tissue. IP: Mouse brain tissue lysate. ICC/IF: Mouse and rat primary neural/glia cells. mlHC: Human cerebrum tissue and human cerebellum

tissue.

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: 59.94% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number EPR23504-106

Isotype IgG

Applications

The Abpromise guarantee

Our Abpromise guarantee covers the use of ab254363 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 |
|-------------|-----------|--|
| ICC/IF | | 1/50. |
| IHC-P | | 1/2000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. |
| IP | | 1/30. |
| WB | | 1/2000. Detects a band of approximately 20, 23 kDa (predicted molecular weight: 30 kDa). |
| mIHC | | Use at an assay dependent concentration. |
| IHC-Fr | | 1/500. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20). |

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Function

This is the major myelin protein from the central nervous system. It plays an important role in the formation or maintenance of the multilamellar structure of myelin.

Involvement in disease

Defects in PLP1 are the cause of leukodystrophy hypomyelinating type 1 (HLD1) [MIM:312080]; also known as Pelizaeus-Merzbacher disease. HLD1 is an X-linked recessive dysmyelinating disorder of the central nervous system in which myelin is not formed properly. It is characterized

clinically by nystagmus, spastic quadriplegia, ataxia, and developmental delay.

Defects in PLP1 are the cause of spastic paraplegia X-linked type 2 (SPG2) [MIM:312920]. SPG2 is characterized by spastic gait and hyperreflexia. In some patients, complicating features include nystagmus, dysarthria, sensory disturbance, mental retardation, optic atrophy.

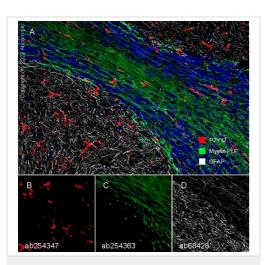
Sequence similarities

Belongs to the myelin proteolipid protein family.

Cellular localization

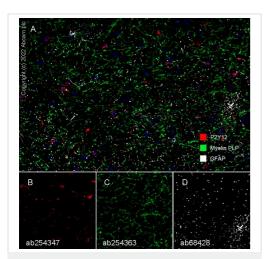
Membrane.

Images



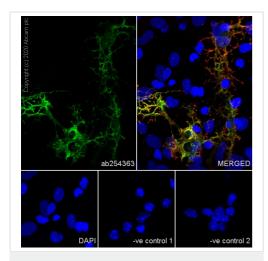
Multiplex immunohistochemistry - Anti-Myelin PLP antibody [EPR23504-106] (ab254363)

Fluorescence multiplex immunohistochemical analysis of the human cerebellum (Formalin/PFA-fixed paraffin-embedded sections). Panel A: merged staining of anti-GFAP (ab68428, gray; Opal™690), anti-Myelin PLP (ab254363, green; Opal™520) and anti-P2Y12 (ab254347 red; Opal™570) on human cerebellum. Panel B: anti-P2Y12 stained on microglial cells. Panel C: anti-Myelin PLP stained on myelin sheaths of oligodendrocytes. Panel D: anti-GFAP stained on astrocytes. Opal Polymer HRP Ms + Rb was used as a secondary antibody. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. The section was incubated in three rounds of staining: in the order of ab68428 (1/50 dilution), ab254363 (1/2000 dilution), and ab254347 (1/1000 dilution) for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Leica SP8 confocal microscope.



Multiplex immunohistochemistry - Anti-Myelin PLP antibody [EPR23504-106] (ab254363)

Fluorescence multiplex immunohistochemical analysis of the human cerebrum (Formalin/PFA-fixed paraffin-embedded sections), Panel A: merged staining of anti-GFAP (ab68428, gray; Opal™690), anti-Myelin PLP (ab254363, green; Opal™520) and anti-P2Y12 (ab254347, red; Opal[™]570) on human cerebrum. Panel B: anti-P2Y12 stained on microglial cells. Panel C: anti-Myelin PLP stained on myelin sheaths of oligodendrocytes. Panel D: anti-GFAP stained on astrocytes. Opal Polymer HRP Ms + Rb was used as a secondary antibody. The immunostaining was performed on a Leica Biosystems BOND[®] RX instrument with an Opal™ 4-color kit. The section was incubated in three rounds of staining: in the order of ab68428 (1/50 dilution), ab254363 (1/2000 dilution), and ab254347 (1/1000 dilution) for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Leica SP8 confocal microscope.

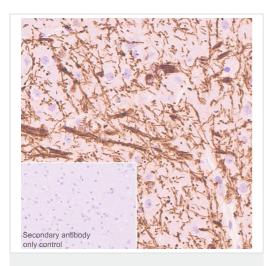


Immunocytochemistry/ Immunofluorescence - Anti-Myelin PLP antibody [EPR23504-106] (ab254363)

Immunocytochemistry analysis of rat primary neural/glia cells labelling Myelin PLP with ab254363 at 1/50 dilution. Cells were fixed with 4% paraformaldehyde. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) (ab150077) at 1/1000 was used as the secondary antibody (green). Cells were counterstained with Anti-Myelin Basic Protein rat monoclonal antibody at 1/100 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) (ab150120) at 1/1000 dilution (red). Nuclear DNA was labelled with DAPI (blue).

Confocal image showing cytoplasmic staining in rat primary glia cells.

Confocal scanning Z step was set as 0.3 µm followed by image processing with maximum Z projection.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Myelin PLP antibody
[EPR23504-106] (ab254363)

Immunohistochemical analysis of paraffin-embedded Mouse cerebrum tissue labeling Myelin PLP with ab254363 at 1/2000 (0.261 ug/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on mouse cerebrum (PMID: 28066178). Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use 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.



Western blot - Anti-Myelin PLP antibody [EPR23504-106] (ab254363)

All lanes : Anti-Myelin PLP antibody [EPR23504-106] (ab254363) at 1/2000 dilution

Lane 1 : Mouse brain tissue lysate

Lane 2 : Mouse liver tissue lysate

Lane 3 : Rat brain tissue lysate

Lane 4: Rat liver tissue lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/100000 dilution

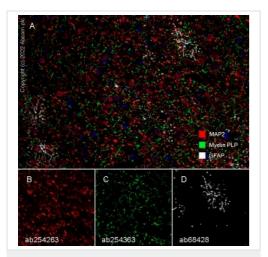
Predicted band size: 30 kDa **Observed band size:** 20,23 kDa

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

The molecular weight observed is consistent with what has been described in the literature (PMID: 9247276).

Negative control: liver (PMID: 2414013).

Exposure time: 37 seconds.



Multiplex immunohistochemistry - Anti-Myelin PLP antibody [EPR23504-106] (ab254363)

Fluorescence multiplex immunohistochemical analysis of human cerebrum tissue (formalin/PFA-fixed paraffin-embedded section). Panel A: merged staining of anti-GFAP (ab68428, gray; Opal™690), anti-Myelin PLP (ab254363, green; Opal™520) and anti-MAP2 (ab254263, red; Opal™570) on human cerebrum tissue. Panel B: anti-MAP2 stained cell body and dendrites of neurons. Panel C: anti-Myelin PLP stained on myelin sheaths of oligodendrocytes. Panel D: anti-GFAP stained on astrocytes. Opal Polymer HRP Ms + Rb was used as a secondary antibody. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. The section was incubated in three rounds of staining: in the order of ab68428 (1/50 dilution), ab254363 (1/2000 dilution), and ab254263 (1/4000 dilution) for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) was used for 20 mins. DAPI (blue) was used as a

nuclear counter stain. Image acquisition was performed with Leica SP8 confocal microscope.

ab254363 MERGED

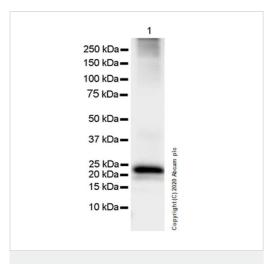
DAPI -ve control 1 -ve control 2

Immunocytochemistry/ Immunofluorescence - Anti-Myelin PLP antibody [EPR23504-106] (ab254363)

Immunocytochemistry analysis of mouse primary neural/glia cells labelling Myelin PLP with ab254363 at 1/50 dilution. Cells were fixed with 4% paraformaldehyde. Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) (ab150077) at 1/1000 was used as the secondary antibody (green). Cells were counterstained with Anti-MAP2 mouse monoclonal antibody (ab11267) at 1/200 dilution and Goat Anti-Mouse lgG H&L (Alexa Fluor® 594) (ab150120) at 1/1000 dilution (red). Nuclear DNA was labelled with DAPI (blue).

Confocal image showing cytoplasmic staining in mouse primary glia cells.

Confocal scanning Z step was set as $0.3~\mu m$ followed by image processing with maximum Z projection.



Western blot - Anti-Myelin PLP antibody [EPR23504-106] (ab254363)

Anti-Myelin PLP antibody [EPR23504-106] (ab254363) at 1/5000 dilution + Human brain tissue lysate at 10 μg

Secondary

VeriBlot for IP secondary antibody(HRP)(<u>ab131366</u>) at 1/1000 dilution

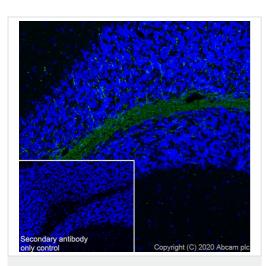
Predicted band size: 30 kDa

Observed band size: 20,23 kDa

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

The molecular weight observed is consistent with what has been described in the literature (PMID: 9247276)

Exposure time: 1 second.

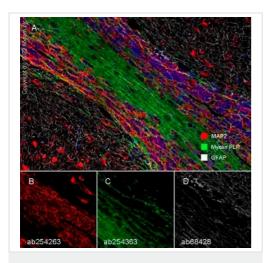


Immunohistochemistry (Frozen sections) - Anti-Myelin PLP antibody [EPR23504-106] (ab254363)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Mouse cerebellum tissue labeling Myelin PLP with ab254363 at 1/500 (1.042 ug/ml) dilution followed by **ab150077** Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) at 1/1000 dilution (Green). Positive staining on mouse cerebellum. The nuclear counterstain was DAPI (Blue).

Secondary antibody control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488)at 1/1000 dilution.

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



Multiplex immunohistochemistry - Anti-Myelin PLP antibody [EPR23504-106] (ab254363)

Fluorescence multiplex immunohistochemical analysis of human cerebellum tissue (formalin/PFA-fixed paraffin-embedded section). Panel A: merged staining of anti-GFAP (ab68428, gray; Opal™690), anti-Myelin PLP (ab254363, green; Opal™520) and anti-MAP2 (ab254263, red; Opal™570) on human cerebellum tissue. Panel B: anti-MAP2 stained cell body and dendrites of neurons. Panel C: anti-Myelin PLP stained on myelin sheaths of oligodendrocytes. Panel D: anti-GFAP stained on astrocytes. Opal Polymer HRP Ms + Rb was used as a secondary antibody. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with an Opal™ 4-color kit. The section was incubated in three rounds of staining: in the order of ab68428 (1/50 dilution), ab254363 (1/2000 dilution), and ab254263 (1/4000 dilution) for 30 mins at room temperature. Each round was followed by a separate fluorescent tyramide signal amplification system. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) was used for 20 mins. DAPI (blue) was used as a nuclear counter stain. Image acquisition was performed with Leica SP8 confocal microscope.



Immunoprecipitation - Anti-Myelin PLP antibody [EPR23504-106] (ab254363)

Myelin PLP was immunoprecipitated from 0.35 mg Mouse brain tissue lysate with ab254363 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab254363 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP)(ab131366) was used at 1/5000 dilution.

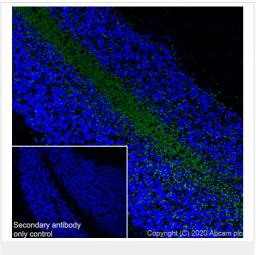
Lane 1: Mouse brain tissue lysate 5 ug

Lane 2: ab254363 IP in Mouse brain tissue lysate

Lane 3: Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab254363 in mouse brain tissue lysate

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

Exposure time: 6 seconds.

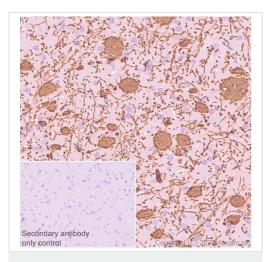


Immunohistochemistry (Frozen sections) - Anti-Myelin PLP antibody [EPR23504-106] (ab254363)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Rat cerebellum tissue labeling Myelin PLP with ab254363 at 1/500 (1.042 ug/ml) dilution followed by **ab150077** Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) at 1/1000 dilution (Green). Positive staining on rat cerebellum. The nuclear counterstain was DAPI (Blue).

Secondary antibody control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488)at 1/1000 dilution.

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

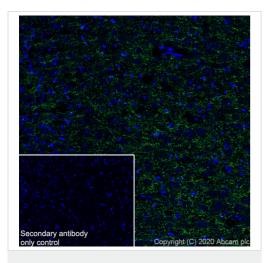


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Myelin PLP antibody
[EPR23504-106] (ab254363)

Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labeling Myelin PLP with ab254363 at 1/2000 (0.261 ug/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on rat cerebrum. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use 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.

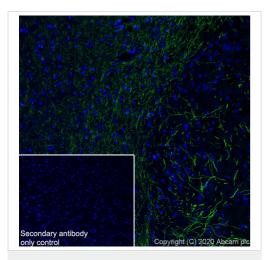


Immunohistochemistry (Frozen sections) - Anti-Myelin PLP antibody [EPR23504-106] (ab254363)

Immunohistochemical analysis of 4% PFA-fixed, 0.2% Triton X-100 permeabilized frozen Rat cerebrum tissue labeling Myelin PLP with ab254363 at 1/500 (1.042 ug/ml) dilution followed by **ab150077** Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) at 1/1000 dilution (Green). Positive staining on rat cerebrum. The nuclear counterstain was DAPI (Blue).

Secondary antibody control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor[®] 488) at 1/1000 dilution.

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

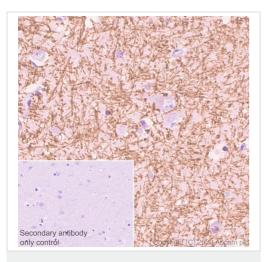


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Secondary antibody control: Secondary antibody is <u>ab150077</u> Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) at 1/1000 dilution.

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

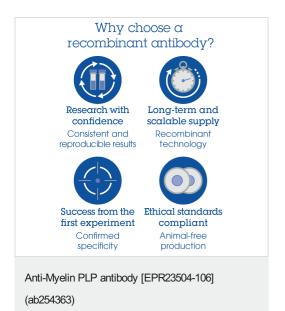


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Myelin PLP antibody
[EPR23504-106] (ab254363)

Immunohistochemical analysis of paraffin-embedded Human cerebrum tissue labeling Myelin PLP with ab254363 at 1/2000 (0.261 ug/ml) dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on human cerebrum (PMID: 29081415). Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use 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.



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