Product datasheet

Anti-Myelin Basic Protein antibody ab40390

Overview

Product name: Anti-Myelin Basic Protein antibody
Description: Rabbit polyclonal to Myelin Basic Protein
Host species: Rabbit
Tested applications: Suitable for: ICC/IF, WB, IHC-FoFr, IHC-Fr, IHC-P
Species reactivity: Reacts with: Mouse, Rat
Immunogen: Synthetic peptide corresponding to Mouse Myelin Basic Protein aa 150 to the C-terminus conjugated to keyhole limpet haemocyanin.
Database link: P04370
(Peptide available as ab40389)

Positive control: IHC-P: Mouse brain tissue; Rat adult brain sagittal tissue. IHC-FoFr: mouse and rat brain WB: Mouse and rat brain tissue lysate. ICC/IF: PC-12 cells.

General notes: For a recombinant version of Myelin Basic Protein antibody () - see ab209328 - clone IGX3421.

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 or -80°C. Avoid freeze / thaw cycle.
Storage buffer: Preservative: 0.02% Sodium Azide
Constituents: 1% BSA, PBS, pH 7.4
Purity: Immunogen affinity purified
Clonality: Polyclonal
Isotype: IgG

Applications

Our Abpromise guarantee covers the use of ab40390 in the following tested applications.
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.
Function
The classic group of MBP isoforms (isoform 4-isoform 14) are with PLP the most abundant protein components of the myelin membrane in the CNS. They have a role in both its formation and stabilization. The smaller isoforms might have an important role in remyelination of denuded axons in multiple sclerosis. The non-classic group of MBP isoforms (isoform 1-isoform 3/Golli-MBPs) may preferentially have a role in the early developing brain long before myelination, maybe as components of transcriptional complexes, and may also be involved in signaling pathways in T-cells and neural cells. Differential splicing events combined with optional post-translational modifications give a wide spectrum of isomers, with each of them potentially having a specialized function. Induces T-cell proliferation.

Tissue specificity
MBP isoforms are found in both the central and the peripheral nervous system, whereas Golli-MBP isoforms are expressed in fetal thymus, spleen and spinal cord, as well as in cell lines derived from the immune system.

Involvement in disease
Note=The reduction in the surface charge of citrullinated and/or methylated MBP could result in a weakened attachment to the myelin membrane. This mechanism could be operative in demyelinating diseases such as chronic multiple sclerosis (MS), and fulminating MS (Marburg disease).

Sequence similarities
Belongs to the myelin basic protein family.

Developmental stage
Expression begins abruptly in 14-16 week old fetuses. Even smaller isoforms seem to be produced during embryogenesis; some of these persisting in the adult. Isoform 4 expression is more evident at 16 weeks and its relative proportion declines thereafter.

Post-translational modifications
Several charge isomers of MBP; C1 (the most cationic, least modified, and most abundant form), C2, C3, C4, C5, C6, C7, C8-A and C8-B (the least cationic form); are produced as a result of optional PTM, such as phosphorylation, deamidation of glutamine or asparagine, arginine citrullination and methylation. C8-A and C8-B contain each two mass isoforms termed C8-A(H), C8-A(L), C8-B(H) and C8-B(L), (H) standing for higher and (L) for lower molecular weight. C3, C4 and C5 are phosphorylated. The ratio of methylated arginine residues decreases during aging, making the protein more cationic. The N-terminal alanine is acetylated (isoform 3, isoform 4, isoform 5 and isoform 6). Arg-241 was found to be 6% monomethylated and 60% symmetrically dimethylated.

Cellular localization
Myelin membrane. Cytoplasmic side of myelin.

Images

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<tr>
<td>ICC/IF</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 5 µg/ml.</td>
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<tr>
<td>WB</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use a concentration of 1 µg/ml. Predicted molecular weight: 33 kDa. Can be blocked with Mouse Myelin Basic Protein peptide (ab40389).</td>
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<tr>
<td>IHC-FoFr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/1000.</td>
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<tr>
<td>IHC-Fr</td>
<td>⭐⭐⭐⭐⭐</td>
<td>Use at an assay dependent concentration.</td>
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<tr>
<td>IHC-P</td>
<td>⭐⭐⭐⭐⭐</td>
<td>1/100 - 1/200. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.</td>
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Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Myelin Basic Protein antibody (ab40390)

This image is courtesy of an Abreview submitted by Mr Carl Hobbs

Representative brain sections with CCm stained for Myelin Basic Protein of amitriptyline-treated mice and respective controls. Scale bar: 100 μm.

For immunohistochemical analysis, animals were sacrificed by isoflurane inhalation (Abbott, Wiesbaden Germany), perfused with ice-cold phosphate-buffered saline (pH 7.4, PBS, Sigma-Aldrich, Steinheim, Germany) followed by 4% paraformaldehyde (PFA, Sigma-Aldrich). Brains were post-fixed in 4% PFA overnight. Thereafter, brains were paraffin-embedded and processed into 5 μm coronal sections between bregma-0.82mm and bregma -1.82mm according to the mouse atlas. Sections were placed on silane-coated slides, de-paraffinized, re-hydrated and heat-unmasked using citrate buffer, pH 6.0, with 3 times 5 minutes microwave cooking at 600W. Slides were treated with 3% H$_2$O$_2$ (Roth, Karlsruhe, Germany) diluted in PBS/17% methanol for 30 minutes to block endogenous peroxidase, followed by 1-hour treatment with 0.2% Casein (w/v; Sigma-Aldrich) + 0.1% Tween 20 (Sigma-Aldrich) + 0.1% Triton X-100 (Sigma-Aldrich) diluted in PBS for 1-hour. Afterwards, sections were exposed overnight at 4°C to primary antibodies diluted in blocking buffer: rabbit polyclonal anti-myelin basic protein (MBP) (1:500, ab40390, Abcam). The next day, sections were incubated with the appropriate secondary antibodies for 1 hour at room temperature.

The extent of de- and remyelination was assessed by quantifying MBP intensity of the CCm.
Western blot - Anti-Myelin Basic Protein antibody (ab40390)

All lanes: Anti-Myelin Basic Protein antibody (ab40390) at 1 µg/ml

Lane 1: Brain (Mouse) Tissue Lysate
Lane 2: Brain (Rat) Tissue Lysate - normal tissue

Lysates/proteins at 10 µg per lane.

Secondary
All lanes: Goat polyclonal to rabbit IgG - H&L - Pre adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

Predicted band size: 33 kDa
Observed band size: 18.23 kDa

why is the actual band size different from the predicted?
Additional bands at: 45 kDa. We are unsure as to the identity of these extra bands.

This antibody was raised against an immunogen that is predicted to recognize isoforms (5, 7, 8, 10 and 13) of Myelin Basic Protein (MBP). The predicted molecular weights of isoforms (5, 7, 8, 10 and 13) are 18.5kDa, 17kDa, 14kDa, 21kDa and 13kDa respectively.

Immunohistochemical detection of Myelin Basic Protein antibody using (ab40390) on PFA perfusion fixed free-floating rat brain sections. Primary antibody used at 1/1000 and incubated for 18 hours @ 20°C in PBS + 0.3 % Triton X100. Secondary antibody: Goat anti-rabbit Alexa Fluor® 488 (1/1000). Immunostaining is observed widely in tracts of axons, as expected. The pictures show the staining obtained at the level of the cerebral cortex, using the objective X5 (left) or X10 (right). The tissues were perfusion fixed with 4% PFA and later postfixed overnight in the same fixative. They were cryoprotected in 30% sucrose and cut using a cryostat.
Immunohistochemical detection of Myelin Basic Protein antibody using (ab40390) on PFA perfusion fixed free-floating rat brain sections. Primary antibody used at 1/1000 and incubated for 18 hours at 20°C in PBST. Secondary antibody: Goat anti-rabbit Alexa Fluor® 488 (1/1000). The sections used came from animals perfusion fixed with Paraformaldehyde 4%, in phosphate buffer 0.2M. Following postfixation in the same fixative overnight, the brains were cryoprotected in sucrose 30% overnight. Brains were then cut using a cryostat and the immunostainings were performed using the 'free floating' technique.

ICC/IF image of ab40390 stained PC12 cells. The cells were 4% formaldehyde fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab40390, 5µg/ml) overnight at +4°C. The secondary antibody (green) was ab96899, DyLight® 488 goat anti-rabbit IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.
ICC/IF image of ab40390 stained PC12 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab40390, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue).

ab40390 staining rat adult brain sagittal tissue section by IHC-P. Sections were formaldehyde fixed and subjected to heat mediated antigen retrieval in citric acid (pH 6) prior to blocking in 1% BSA for 10 minutes at RT. The primary antibody was diluted 1/100 and incubated with the sample for 16 hours. A biotinylated goat anti-rabbit IgG antibody, diluted 1/300, was used as the secondary.

Impression:

Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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