## abcam

### Product datasheet

# Anti-Myelin Basic Protein antibody [IGX3421] ab209328

★★★★★ 9 Abreviews 8 References 9 Images

#### Overview

Product name	Anti-Myelin Basic Protein antibody [IGX3421]	
Description	Human monoclonal [IGX3421] to Myelin Basic Protein	
Host species	Human	
Tested applications	Suitable for: ELISA, WB, ICC/IF, IHC-P	
Species reactivity	Reacts with: Mouse, Rat, Human, Recombinant fragment	
	Predicted to work with: Horse, Cow, Cat, Dog, Pig, Chimpanzee, Macaque monkey 🛛 🔺	
Immunogen	Full length native protein (purified). This information is proprietary to Abcam and/or its suppliers.	
Positive control	WB: Human, mouse and rat brain tissue lysate. Myelin Basic Protein (recombinant protein) IHC-P: Mouse, rat and human brain tissue (Hippocampus) ICC/IF: SK-N-SH cells	
General notes	This product was made using synthetic libraries and phage display technology.	
	This antibody is a recombinant antibody. Human monoclonal antibody.	
	Example of usage (reference):	
	Spatiotemporal Dynamics of Molecular Pathology in Amyotrophic Lateral Sclerosis	
	Silas Maniatis, Tarmo Aijo, Sanja Vickovic, Catherine Braine, Kristy Kang, Annelie Mollbrink, Zaneta Andrusivova, Sami Saarenpaa, Gonzalo Saiz-Castro, Miguel Cuevas, Aaron Watters, Joakim Lundeberg, Richard Bonneau, Hemali Phatnani doi: https://doi.org/10.1101/389270	

#### **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	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 0.05% BSA, 40% Glycerol (glycerin, glycerine)
Purity	Protein A purified

Clonality	Monoclonal
Clone number	IGX3421
lsotype	lgG1

#### Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab209328 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
ELISA		Use at an assay dependent concentration.
WB	<b>★ ★ ★ ★ ☆</b> <u>(2)</u>	Use a concentration of 0.25 - 1 µg/ml. Detects a band of approximately 20,17 kDa (predicted molecular weight: 33 kDa).
ICC/IF		Use a concentration of 5 $\mu\text{g/ml.}$ This product gave a positive signal in SKNSH cells fixed with 4% formaldehyde
IHC-P	<b>★ ★ ★ ★ ★ (6)</b>	Use a concentration of 0.5 - 1 $\mu$ g/ml. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

#### Target

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 chronical 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	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

#### modifications

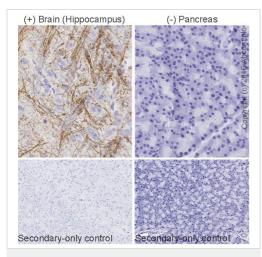
optional PTM, such as phosphorylation, deamidation of glutamine or asparagine, arginine citrullination and methylation. C8-A and C8-B contain each two mass isoforms termed C8-A(H), C8-A(L), C8-B(H) and C8-B(L), (H) standing for higher and (L) for lower molecular weight. C3, C4 and C5 are phosphorylated. 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.

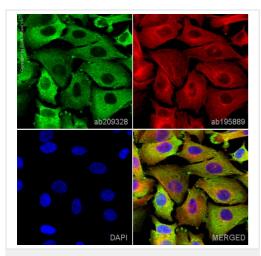
**Cellular localization** 

Myelin membrane. Cytoplasmic side of myelin.

#### Images



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Myelin Basic Protein antibody [IGX3421] (ab209328)



Immunocytochemistry/ Immunofluorescence - Anti-Myelin Basic Protein antibody [IGX3421] (ab209328)

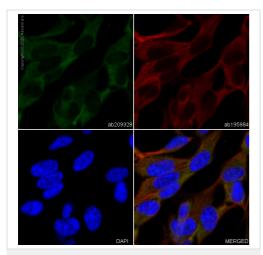
IHC image of Myelin Basic Protein staining in a section of formalinfixed paraffin-embedded normal rat brain and normal rat pancreas, performed on a Leica BOND<sup>TM</sup>. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6, epitope retrieval solution 1) for 20 minutes. The section was then incubated with ab209328, 1/1000 dilution, for 15 minutes at room temperature.

An HRP-conjugated goat anti-Human IgG secondary was used for 15 minutes at room temperature. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

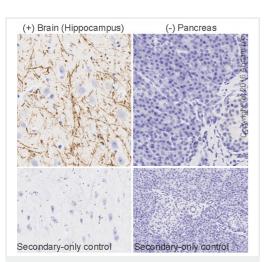
ab209328 staining Myelin Basic Protein in SK-N-SH (Human neuroblastoma cell line) cells. The cells were fixed with 4% formaldehyde (10 minutes), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal donkey serum/0.3M glycine in 0.1% PBS-Tween for 1 hour. The cells were then incubated overnight at +4°C with ab209328 at a 5 µg/ml concentration, then detected with a donkey anti-human (Alexa Fluor<sup>®</sup> 488) secondary antibody at a 1/2000 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue), and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor<sup>®</sup> 594), at a 1/250 dilution (shown in red).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-Myelin Basic Protein antibody [IGX3421] (ab209328) ab209328 staining Myelin Basic Protein in SHSY5Y cells. The cells were fixed with 100% methanol (5 minutes), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal donkey serum/0.3M glycine in 0.1% PBS-Tween for 1 hour. The cells were then incubated overnight at +4°C with ab209328 at a 5  $\mu$ g/ml concentration, then detected with a donkey anti-human (Alexa Fluor<sup>®</sup> 488) secondary antibody at a 1/1000 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue), and **ab195884**, Rat monoclonal to alpha Tubulin (Alexa Fluor<sup>®</sup> 647), at a 1/250 dilution (shown in red).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

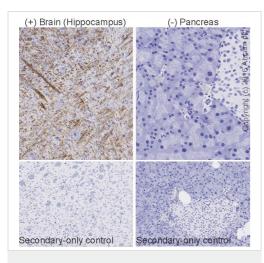


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Myelin Basic Protein antibody [IGX3421] (ab209328) IHC image of Myelin Basic Protein staining in a section of formalinfixed paraffin-embedded normal human hippocampus and normal human pancreas<sup>\*</sup>, performed on a Leica BOND<sup>™</sup>. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6, epitope retrieval solution 1) for 20 minutes. The section was then incubated with ab209328, 1/1000 dilution, for 15 minutes at room temperature.

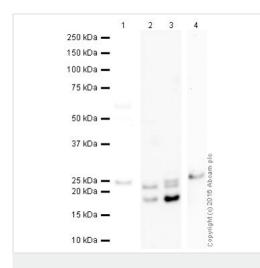
An HRP-conjugated goat anti-Human IgG secondary was used for 15 minutes at room temperature. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

\*Human pancreas tissue was obtained from the Human Research Tissue Bank, supported by the NIHR Cambridge Biomedical Research Centre



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Myelin Basic Protein antibody [IGX3421] (ab209328)



Western blot - Anti-Myelin Basic Protein antibody [IGX3421] (ab209328) IHC image of Myelin Basic Protein staining in a section of formalinfixed paraffin-embedded normal mouse brain and normal mouse pancreas, performed on a Leica BOND<sup>™</sup>. The section was pretreated using heat mediated antigen retrieval with sodium citrate buffer (pH 6, epitope retrieval solution 1) for 20 minutes. The section was then incubated with ab209328, 1/1000 dilution, for 15 minutes at room temperature.

An HRP-conjugated goat anti-Human IgG secondary was used for 15 minutes at room temperature. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

**All lanes :** Anti-Myelin Basic Protein antibody [IGX3421] (ab209328) at 0.25 µg/ml

Lane 1 : Human brain tissue lysate - total protein (<u>ab29466</u>) at 10 µg

Lane 2 : Mouse brain tissue lysate at 10 µg

Lane 3 : Rat brain tissue lysate at 10 µg

Lane 4: Myelin Basic Protein (Recombinant protein) at 0.1 µg

#### Secondary

**All lanes :** HRP conjugated Goat Anti-Human lgG (H+L) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 33 kDa Observed band size: 18,23,24 kDa

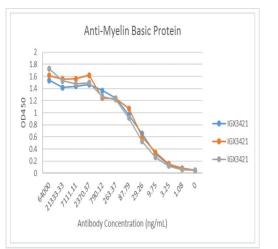
#### Exposure time :

Lane 1:30 seconds.

Lanes 2-3 : 2 minutes.

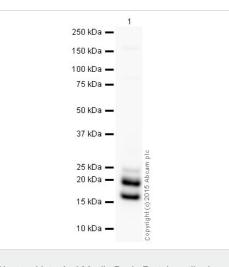
Lane 4:8 minutes.

This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% milk before being incubated with ab209328 overnight at 4°C. Antibody binding was visualised using ECL development solution **ab133406**.



ELISA using ab209328 for 16 hours at 4°C. <u>ab7153</u> goat anti human was used as a secondary at a 1/5000 dilution for 1 hour at Room Temperature.

ELISA - Anti-Myelin Basic Protein antibody [IGX3421] (ab209328)



Western blot - Anti-Myelin Basic Protein antibody [IGX3421] (ab209328) Anti-Myelin Basic Protein antibody [IGX3421] (ab209328) at 1  $\mu$ g/ml + Mouse brain tissue lysate at 10  $\mu$ g

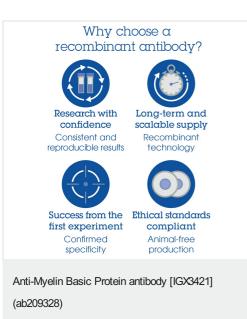
#### Secondary

HRP conjugated Goat Anti-Human IgG (H+L) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 33 kDa Observed band size: 20, 17 kDa This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a Nitrocellulose membrane at 30V for 70 minutes. The membrane was then blocked for an hour using 3% milk before being incubated with ab209328 overnight at 4°C. Antibody binding was visualised using ECL development solution **ab133406**.



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