Anti-MAG Antibody [PSH02-41]

HA721818

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Tissue, IP, IHC-Fr
Molecular Wt:	Predicted band size: 69 kDa
Clone number:	PSH02-41

Description:	Adhesion molecule that mediates interactions between myelinating cells and neurons by binding to neuronal sialic acid-containing gangliosides and to the glycoproteins RTN4R and RTN4RL2 (By similarity). Not required for initial myelination, but seems to play a role in the maintenance of normal axon myelination. Protects motoneurons against apoptosis, also after injury; protection against apoptosis is probably mediated via interaction with neuronal RTN4R and RTN4RL2. Required to prevent degeneration of myelinated axons in adults; this probably depends on binding to gangliosides on the axon cell membrane (By similarity). Negative regulator of neurite outgrowth; in dorsal root ganglion neurons the inhibition is mediated primarily via binding to neuronal RTN4R or RTN4RL2 and to a lesser degree via binding to neuronal gangliosides. In cerebellar granule cells the inhibition is mediated primarily via binding to neuronal gangliosides. In sensory neurons, inhibition of neurite extension depends only partially on RTN4R, RTN4RL2 and gangliosides. Inhibits axon longitudinal growth (By similarity). Inhibits axon outgrowth by binding to RTN4R (By similarity). Preferentially binds to alpha-2,3-linked sialic acid. Binds ganglioside Gt1b (By similarity).
Immunogen:	Recombinant protein within human MAG aa 1- 536 / 626.
Positive control:	Mouse brain(no heat) tissue lysate, mouse cerebellum tissue lysate, rat brain(no heat) tissue lysate, rat cerebellum tissue lysate, human brain tissue, human cerebellum tissue, mouse brain tissue, mouse cerebellum tissue, mouse hippocampus tissue, rat brain tissue, rat cerebellum tissue, rat hippocampus tissue.
Subcellular location:	Cell membrane.
Database links:	SwissProt: P20916 Human | P20917 Mouse | P07722 Rat
Recommended Dilutions:	
WB	1:2,000
IHC-P	1:2,000-1:5,000
IF-Tissue	1:200
IP	1-2μg/sample
IHC-Fr	1:500
Storage Buffer:	PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

Images

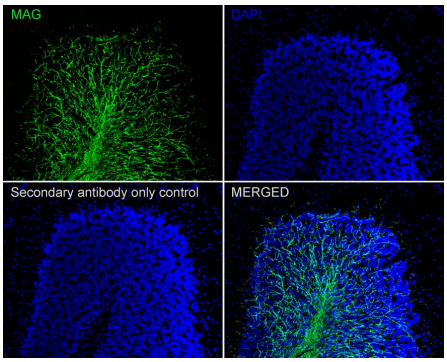

Fig1: Immunofluorescence analysis of frozen mouse cerebellum tissue with Rabbit anti-MAG antibody (HA721818) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721818, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

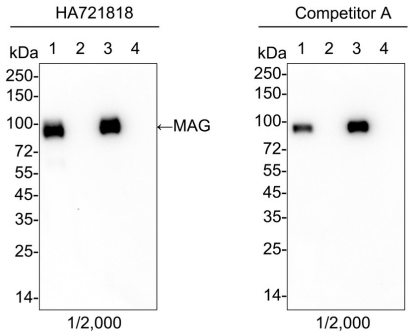

Fig2: Western blot analysis of MAG on different lysates with Rabbit anti-MAG antibody (HA721818) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution.

Lane 1: Mouse cerebellum tissue lysate
Lane 2: Mouse liver tissue lysate (negative)
Lane 3: Rat cerebellum tissue lysate
Lane 4: Rat liver tissue lysate (negative)

Lysates/proteins at 20 µg/Lane.

Predicted band size: 69 kDa
Observed band size: 100 kDa

Exposure time: 2 minutes; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721818) at 1/2,000 dilution and competitor's antibody at 1/2,000 dilution were used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

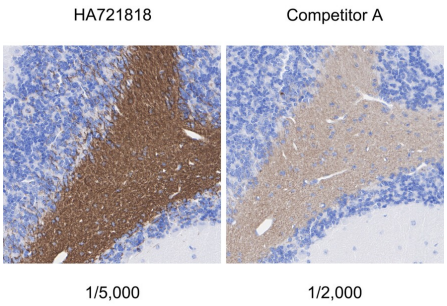

Fig3: Immunohistochemical analysis of paraffin-embedded mouse cerebellum tissue with Rabbit anti-MAG antibody (HA721818) at 1/5,000 dilution and competitor's antibody at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721818) at 1/5,000 dilution and competitor's antibody at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

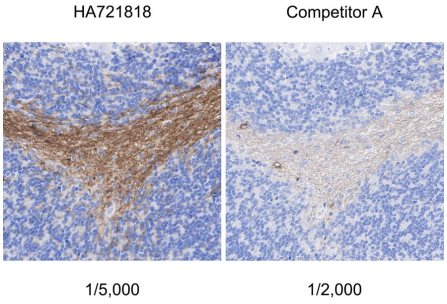

Fig4: Immunohistochemical analysis of paraffin-embedded rat cerebellum tissue with Rabbit anti-MAG antibody (HA721818) at 1/5,000 dilution and competitor's antibody at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721818) at 1/5,000 dilution and competitor's antibody at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

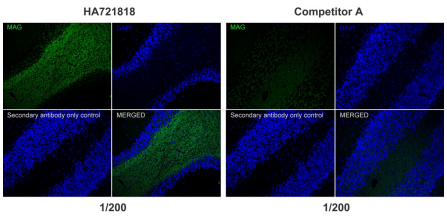

Fig5: Immunofluorescence analysis of paraffin-embedded mouse cerebellum tissue labeling MAG with Rabbit anti-MAG antibody (HA721818) at 1/200 dilution and competitor's antibody at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721818, green) at 1/200 dilution and competitor's antibody at 1/200 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

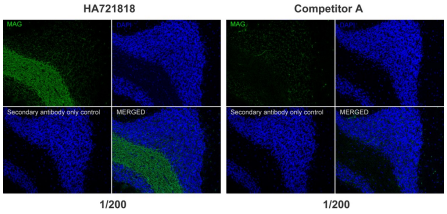

Fig6: Immunofluorescence analysis of paraffin-embedded rat cerebellum tissue labeling MAG with Rabbit anti-MAG antibody (HA721818) at 1/200 dilution and competitor's antibody at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721818, green) at 1/200 dilution and competitor's antibody at 1/200 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

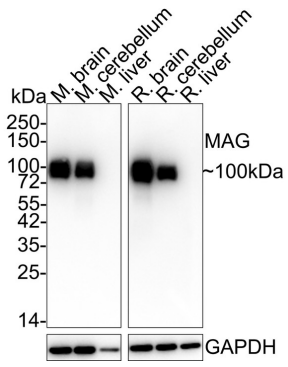

Fig7: Western blot analysis of MAG on different lysates with Rabbit anti-MAG antibody (HA721818) at 1/2,000 dilution.

Lane 1: Mouse brain(no heat) tissue lysate (20 µg/Lane)
Lane 2: Mouse cerebellum tissue lysate (20 µg/Lane)
Lane 3: Mouse liver tissue lysate (negative) (20 µg/Lane)
Lane 4: Rat brain(no heat) tissue lysate (20 µg/Lane)
Lane 5: Rat cerebellum tissue lysate (20 µg/Lane)
Lane 6: Rat liver tissue lysate (negative) (20 µg/Lane)

Predicted band size: 69 kDa
Observed band size: 100 kDa

Exposure time: 42 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA721818) at 1/2,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

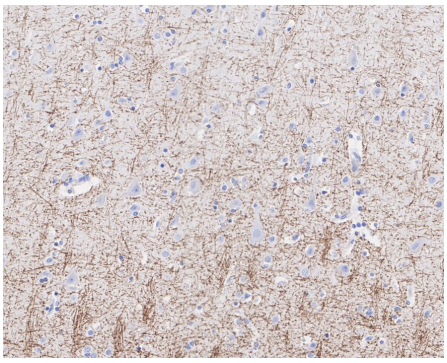

Fig8: Immunohistochemical analysis of paraffin-embedded human brain tissue with Rabbit anti-MAG antibody (HA721818) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721818) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

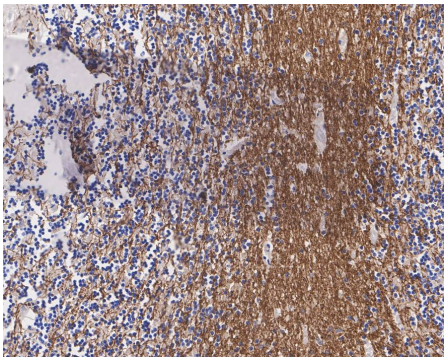

Fig9: Immunohistochemical analysis of paraffin-embedded human cerebellum tissue with Rabbit anti-MAG antibody (HA721818) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721818) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

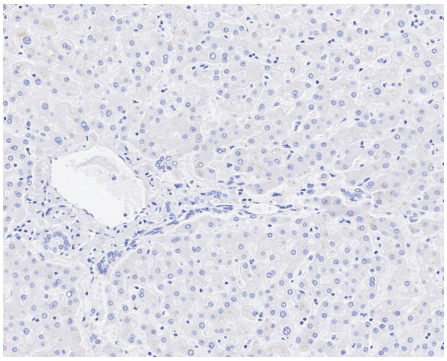

Fig10: Immunohistochemical analysis of paraffin-embedded human liver (negative) tissue with Rabbit anti-MAG antibody (HA721818) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721818) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

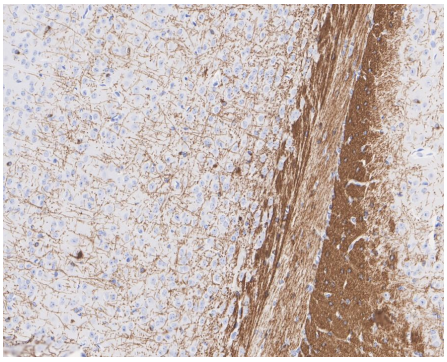

Fig11: Immunohistochemical analysis of paraffin-embedded mouse brain tissue with Rabbit anti-MAG antibody (HA721818) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721818) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

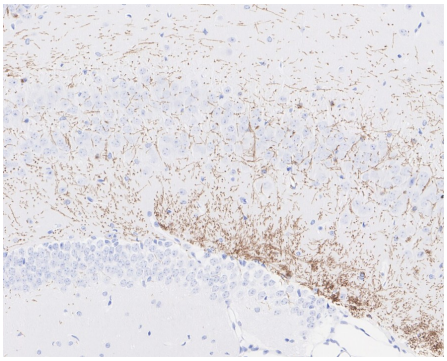

Fig12: Immunohistochemical analysis of paraffin-embedded mouse hippocampus tissue with Rabbit anti-MAG antibody (HA721818) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721818) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

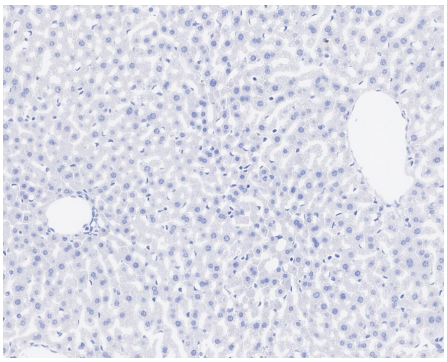

Fig13: Immunohistochemical analysis of paraffin-embedded mouse liver (negative) tissue with Rabbit anti-MAG antibody (HA721818) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721818) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

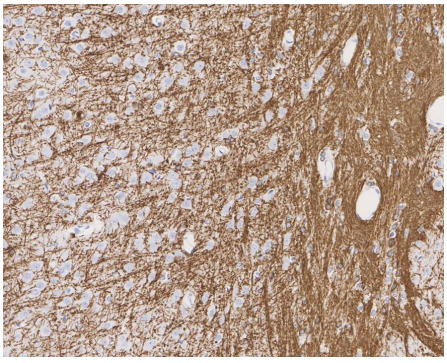

Fig14: Immunohistochemical analysis of paraffin-embedded rat brain tissue with Rabbit anti-MAG antibody (HA721818) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721818) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

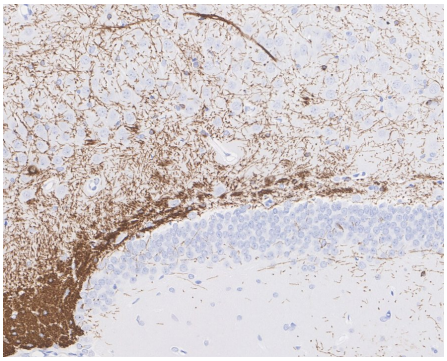

Fig15: Immunohistochemical analysis of paraffin-embedded rat hippocampus tissue with Rabbit anti-MAG antibody (HA721818) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721818) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

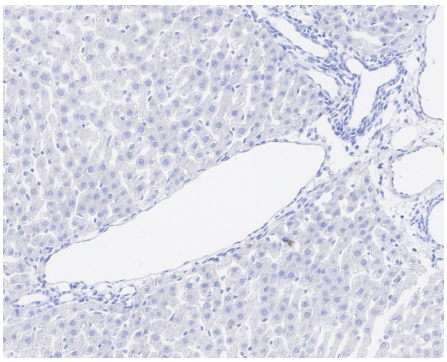

Fig16: Immunohistochemical analysis of paraffin-embedded rat liver (negative) tissue with Rabbit anti-MAG antibody (HA721818) at 1/2,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA721818) at 1/2,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

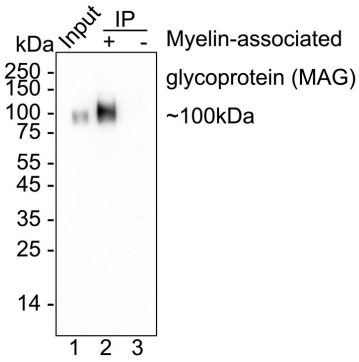

Fig17: MAG was immunoprecipitated from 0.2 mg mouse brain tissue lysate with HA721818 at 2 µg/25 µl agarose. Western blot was performed from the immunoprecipitate using HA721818 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: Mouse brain tissue lysate (input)
Lane 2: HA721818 IP in mouse brain tissue lysate
Lane 3: Rabbit IgG instead of HA721818 in mouse brain tissue lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 32 seconds; ECL: K1801

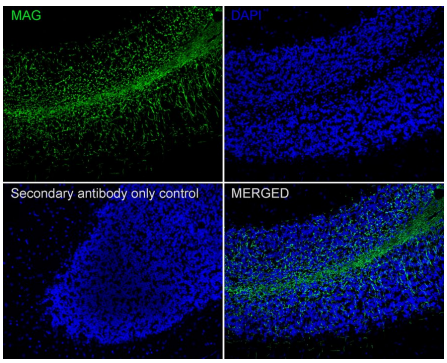

Fig18: Immunofluorescence analysis of frozen rat cerebellum tissue with Rabbit anti-MAG antibody (HA721818) at 1/500 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven. The tissues were blocked in 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (HA721818, green) at 1/500 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

Background References

1. Zis P, Rao DG, Hoggard N, et al. Anti-MAG associated cerebellar ataxia and response to rituximab. J Neurol. 2018 Jan;265(1):115-118.
2. Quarles RH. Myelin-associated glycoprotein (MAG): past, present and beyond. J Neurochem. 2007 Mar;100(6):1431-48.