10320 Camino Santa Fe, Suite G
San Diego, CA 92121
Tel: 858.875.1900 Fax: 858.875.1999

Anti-MAG Picoband™ Antibody (monoclonal, 2G11)

Catalog # ABO15007

Specification

Anti-MAG Picoband™ Antibody (monoclonal, 2G11) - Product Information

Application	**WB, IHC, IF, FC**
Primary Accession	P20916
Host	**Mouse**
Isotype	**Mouse IgG2a**
Reactivity	**Rat, Human, Mouse**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-MAG Picoband™ Antibody (monoclonal, 2G11) . Tested in Flow Cytometry, IF, IHC, WB applications. This antibody reacts with Human, Mouse, Rat.

Reconstitution
Add 0.2ml of distilled water will yield a concentration of 500ug/ml.

Anti-MAG Picoband™ Antibody (monoclonal, 2G11) - Additional Information

Gene ID 4099

Other Names
Myelin-associated glycoprotein, Siglec-4a, MAG, GMA

Calculated MW
100 kDa KDa

Application Details
Western blot, 0.1-0.25 µg/ml, Mouse, Rat
 Immunohistochemistry (Paraffin-embedded Section), 2-5 µg/ml, Mouse,Rat
 Immunofluorescence, 5 µg/ml, Rat
 Flow Cytometry, 1-3 µg/1x10^6 cells, Human

Contents
Each vial contains 4mg Trehalose, 0.9mg NaCl and 0.2mg Na2HPO4.

Immunogen
E.coli-derived human MAG recombinant protein (Position: E34-R605).

Purification
Immunogen affinity purified.

Storage	**Store at -20˚C for one year from date of receipt. After reconstitution, at 4˚C for one month. It can also be aliquotted and stored frozen at -20˚C for six months. Avoid repeated freeze-thaw cycles.**

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Anti-MAG Picoband™ Antibody (monoclonal, 2G11) - Protein Information

Name MAG

Synonyms GMA

Function
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).

Cellular Location
Cell membrane; Single-pass type I membrane protein Membrane raft {ECO:0000250|UniProtKB:P07722}

Tissue Location
Both isoform 1 and isoform 2 are detected in myelinated structures in the central and peripheral nervous system, in periaxonal myelin and at Schmidt-Lanterman incisures (PubMed:6200494, PubMed:9495552). Detected in optic nerve, in oligodendroglia and in periaxonal myelin sheaths (PubMed:6200494). Detected in compact myelin (at protein level) (PubMed:6200494). Both isoform 1 and isoform 2 are detected in the central and peripheral nervous system (PubMed:9495552)

Anti-MAG Picoband™ Antibody (monoclonal, 2G11) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

Anti-MAG Picoband™ Antibody (monoclonal, 2G11) - Images

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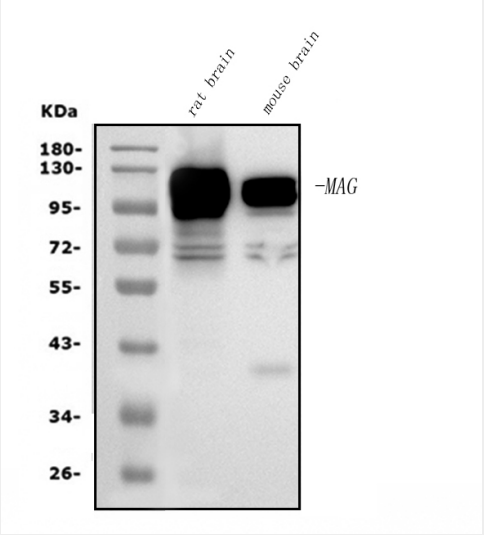

Figure 1. Western blot analysis of MAG using anti-MAG antibody (M03019).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 50ug of sample under reducing conditions.
Lane 1: rat brain tissue lysates,
Lane 2: mouse brain tissue lysates.
After Electrophoresis, proteins were transferred to a Nitrocellulose membrane at 150mA for 50-90 minutes. Blocked the membrane with 5% Non-fat Milk/ TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-MAG antigen affinity purified monoclonal antibody (Catalog # M03019) at 0.25 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for MAG at approximately 100KD. The expected band size for MAG is at 100KD.

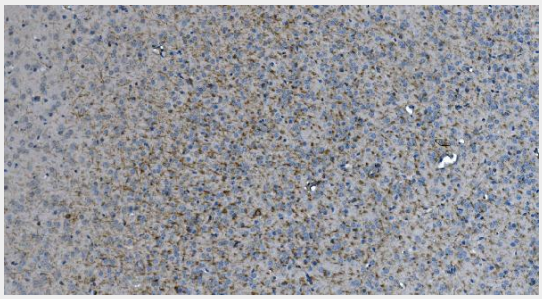

Figure 2. IHC analysis of MAG using anti-MAG antibody (M03019).
MAG was detected in paraffin-embedded section of mouse brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-MAG Antibody (M03019) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

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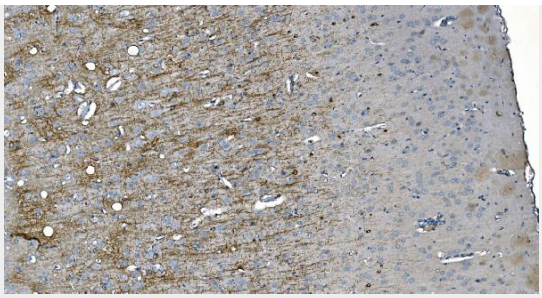

Figure 3. IHC analysis of MAG using anti-MAG antibody (M03019).
MAG was detected in paraffin-embedded section of rat brain tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-MAG Antibody (M03019) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

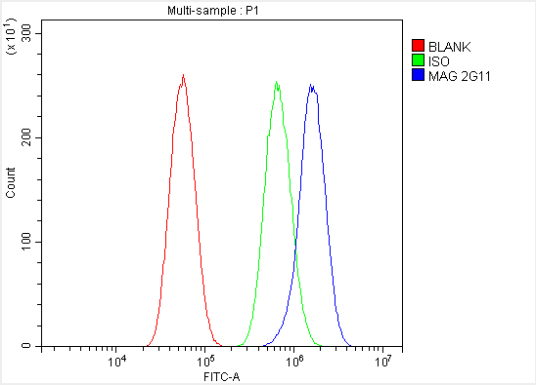

Figure 4. Flow Cytometry analysis of U87 cells using anti-MAG antibody (M03019).
Overlay histogram showing U87 cells stained with M03019 (Blue line).The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-MAG Antibody (M03019, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

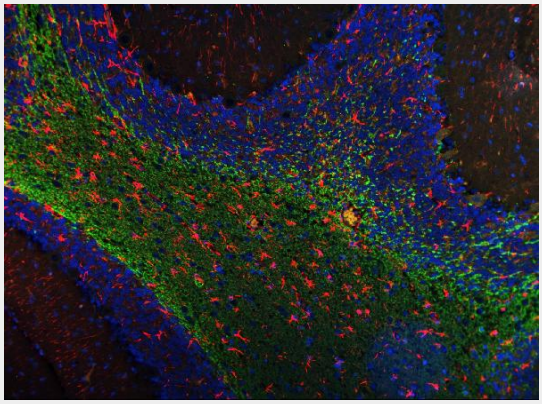

Figure 5. IF analysis of GFAP and MAG using anti-GFAP antibody and anti-MAG antibody (M03019).
GFAP and MAG was detected in a paraffin-embedded section of rat cerebellum tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 5 µg/mL rabbit anti-GFAP antibody and mouse anti-MAG antibody (M03019) overnight at 4°C.

DyLight®594 Conjugated Goat Anti-Rabbit IgG (BA1142), DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

Anti-MAG Picoband™ Antibody (monoclonal, 2G11) - Background

MAG (Myelin-associated glycoprotein),also known as SIGLEC4A, is a cell membrane glycoprotein that is a member of the SIGLEC family of proteins and is a functional ligand of the NOGO-66 receptor, NgR. It is though to be involved in the process of myelination. MAG is a sialic acid-binding SIGLEC protein and is a functional ligand for the NOGO receptor.The MAG gene is mapped on 19q13.12. Cleavage of GPI-linked proteins from axons protects growth cones from MAG-induced collapse, and dominant-negative NgR eliminates MAG inhibition of neurite outgrowth. MAG-resistant embryonic neurons were rendered MAG-sensitive by expression of NgR. MAG binds specifically to an NgR-expressing cell line in a GPI-dependent and sialic acid-independent manner. Experiments blocking NgR from interacting with MAG prevented inhibition of neurite outgrowth by MAG. In cultured human embryonic kidney (HEK) cells expressing the NOGO receptor, p75 (NTR) was required for MAG-induced intracellular calcium elevation.