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AM26432AF-N Monoclonal Antibody to GPM6A - Azide Free

Alternate names:	Neuronal membrane glycoprotein M6-a
Quantity:	0.1 mg
Concentration:	1.0 mg/ml
Background:	M6 is a membrane glycoprotein that is abundantly expressed on central neurons in the CHS. Two distinct murine M6 cDNA (designated M6a and M6b) whose deduced amino acid sequences are remarkably similar to that of the major CNS myelin protein PLP/DM20 are known. PLP expression is limited to the white matter. M6a appears in post-mitotic neurons of the brain and spinal cord as early as embryonic day 10, and later in the hippocampus, cerebral cortex, and the granule cells of the cerebellum. In contrast, M6b is expressed at early embryonic stage in the ventricular zone of the spinal cord, and later during development in both neurons and glia.
Uniprot ID:	P35802
NCBI:	NP_705809.1
GeneID:	234267
Host / Isotype:	Rat / IgG2a
Recommended Isotype Controls:	SM26A
Clone:	321
Immunogen:	Mouse M6a expressing cells
Format:	**State:** Liquid Ig fraction **Purification:** Protein G Agarose **Buffer System:** PBS containing 50% glycerol, pH 7.2 **Preservatives:** None
Applications:	**Immunohistochemistry on Frozen Sections:** 1-10 µg/ml. **Flow Cytometry:** 5-10 µg/ml (final concentration). For details see protocols below. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Specificity:	This antibody reacts with Mouse M6a. Other species not tested.
Add. Information:	This product was originally produced by MBL International.
Storage:	Store (in aliquots) at -20°C. Avoid repeated freezing and thawing. Shelf life: one year from despatch.
General Readings:	1. Alfonso, J., et al., Proc. Natl. Acad. Sci. USA 102, 17196-17201 (2005).
Protocols:	Flow cytometric analysis for floating cells We usually use Fisher tubes or equivalents as reaction tubes for all steps described below. 1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN3].

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Material Safety Datasheets are available at www.acris-antibodies.com or on request.

2) Resuspend the cells with washing buffer (5x10e6 cells/mL).
3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25oC). Remove supernatant by careful aspiration.
4) Add 10 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 20 µL of the anti-mouse M6a monoclonal antibody (clone 321) (5-10 µg/mL) diluted with the washing buffer. Mix well and incubate for 30 minutes at room temperature.
6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
7) Add 20 µL of 1:100 FITC conjugated anti-rat IgG diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
8) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.
(Positive controls for flow cytometry ; LO, transfectant)

Immunohistochemical staining for frozen sections
1) Animals were transcardially perfused with 4% paraformaldehyde (PFA) in 0.1 M PBS pH 7.2.
2) Brains were removed and immediately frozen in liquid nitrogen.
3) Coronal cryosections (40 µm) were washed in 0.5% Triton X-100 in PBS and were immersed for 1 hour at room temperature in blocking buffer (PBS containing 5% normal rabbit serum and 0.5% Triton X-100).
4) Tip off the blocking buffer, wipe gently around each section and cover tissues with primary antibody diluted with PBS as suggest in the APPLICATIONS.
5) Incubate the sections for 2 days at 4 oC.
6) Wash the slides 3 times in PBS for 5 minutes each.
7) Wipe gently around each section and cover tissues with Biotin-conjugated anti-rat Ig. Incubate for 4 hours at room temperature. Wash as in step 6).
8) Wipe gently around each section and cover tissues with Streptavidin-horseradish peroxidase. Incubate for 2 hours at room temperature. Wash as in step 6).
9) Visualize by reacting for 10-20 minutes with substrate solution containing 7.5 mg DAB, 40 µL of 30% H_2O_2 in 150 mL PBS.
*DAB is a suspected carcinogen and must be handled with care. Always wear gloves.
10) Wash the slides in water for 5 minutes.
11) Counter stain in hematoxylin for 1-5 minute, wash the slides 3 times in water for 5 minutes each, and then immerse the slides in PBS for 5 minutes. Dehydrate by immersing in Ethanol 3 times for 3 minutes each, followed by immersing in Xylene 3 times for 3 minutes each.
12) Now ready for mounting.
(Positive control for immunohistochemistry; rat hippocampus)

Pictures:

Flow cytometric analysis of Mouse M6a expression in LO cells. Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the rezction of AM26432AF-N to the cells.