For Research Use Only.
Not for use in diagnostic procedures.

MONOCLONAL ANTIBODY

Anti-M6a (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D055-3	321	Rat IgG2a κ	100 µL	1 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.

SOURCE: This antibody was purified from hybridoma (clone 321) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3X with Wister rat splenocyte immunized with mouse M6a expressing cells.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with mouse M6a on flow cytometry.

APPLICATIONS:
Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; 1-10 µg/mL
This antibody is available for the frozen section.
Immunocytochemistry ; Not tested
Flow Cytometry; 5-10 µg/mL (final concentration)

Detailed procedure is provided in the following **PROTOCOLS**.

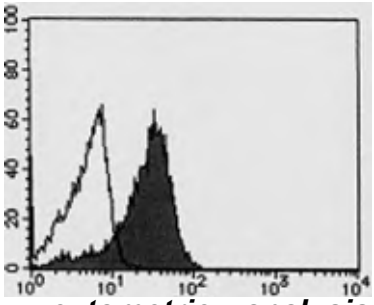
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Not tested	LO, transfectant	Not tested
Reactivity on FCM		+	

INTENDED USE:
For research use only. Not for clinical diagnosis.

REFERENCES:
1) Zhao, J., *et al*., *Mol. Vis*. **14**, 1623-1630 (2008) [IHC]
2) Cooper, B., *et al., Brain Res*. **1197**, 1-12 (2008)
3) Alfonso, J., *et al*., *PNAS* **102**, 17196-17201 (2005)

Clone 321 is used in this reference.



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 reaction of D055-3 to the cells.*

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.

PROTOCOLS:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3].
*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
2) Resuspend the cells with washing buffer ($5x10^6$ 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 (20~25°C). Remove supernatant by careful aspiration.
4) Add 10 µL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.

5) Add 20 µL of the primary antibody as suggested in the **APPLICATIONS** 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 (BD Pharmingen: code no. 554016) 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 suggested in the **APPLICATIONS**.
5) Incubate the sections for 2 days at 4°C.
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 (DaKo JAPAN, code no. E0464). 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 suspect 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)

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