

MOUSE ANTI-OLIGODENDROCYTE MARKER O4 MONOCLONAL ANTIBODY

CATALOG NUMBER: MAB345 (formerly Roche Catalog Number 1518925)

LOT NUMBER:

QUANTITY: 50 µg

CONCENTRATION: 1.05 mg/mL

SPECIFICITY: Oligodendrocyte marker O4.

BACKGROUND: Oligodendrocytes and astrocytes are derived from common precursor cells, glioblasts (Sommer, 1981). O-Antigens are sulfatides, which function as differentiation markers on the surface of oligodendrocytes of the central nervous system. O4 is formed postnatally (Schachner, 1981) and is a marker for cell bodies and processes of oligodendrocytes types I and II (hairy eyeball type). During oligodendrocyte differentiation, O4 occurs in pro-oligodendrocytes, but not in O-2A-progenitor cells. O4 occurs from day 3 onwards in cell cultures of embryonic mouse brain. Anti-O4 can be used in myelinisation, demyelination and remyelination studies and in regeneration experiments.

IMMUNOGEN: Homogenate of white matter of corpus callosum from bovine brain.

ISOTYPE: IgM

CLONE: 81. This antibody is commonly referred to in the literature as monoclonal antibody O4.

APPLICATIONS: Immunohistochemistry: 10-20 µg/mL on unfixed, shock frozen tissue.
Immunocytochemistry: 10-20 µg/mL on cells fixed with 4% paraformaldehyde.
Note: O4 is a sulfatide, which can be dissolved out of the membrane by organic solvents; acetone and methanol should not be used for fixation.
Optimal working dilutions must be determined by the end user.

SPECIES REACTIVITIES: Human, mouse, rat and chicken.

FORMAT: Purified immunoglobulin.

PRESENTATION: Liquid in 0.05M Potassium phosphate buffer, 0.3M NaCl, pH 8.0 with and 0.05% sodium azide.

STORAGE/HANDLING: Maintain at 2-8°C in undiluted aliquots for up to 6 months after date of receipt.

REFERENCES:

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- 21) Goetz, A., *et al.*, *PNAS* (2006) **103**:11063-11068.

For research use only; not for use as a diagnostic.

Important Note: *During shipment, small volumes of product will occasionally become entrapped in the seal of the product vial. For products with volumes of 200 μ L or less, we recommend gently tapping the vial on a hard surface or briefly centrifuging the vial in a tabletop centrifuge to dislodge any liquid in the container's cap.*

Unless otherwise stated in our catalog or other company documentation accompanying the product(s), our products are intended for research use only and are not to be used for any other purpose, which includes but is not limited to, unauthorized commercial uses, *in vitro* diagnostic uses, *ex vivo* or *in vivo* therapeutic uses or any type of consumption or application to humans or animals.

APPLICATION NOTES FOR MAB345

Immunohistochemistry

1. Prepare sections from unfixed, shock frozen tissue. The sections should be 4-5 μm thick. Place the sections on microscope slides.
2. Wash the slide three times for 5 min. each in PBS at room temperature.
3. Block the non-specific binding sites by incubating the sections in a human chamber with 5% FCS at room temperature for 30 minutes.
4. Wash the slides as described in step 2.
5. Cover the sections with a sufficient amount of MAB345 (10-20 $\mu\text{g}/\text{mL}$ in PBS) and incubate in a humid chamber at 37°C for one hour.
6. Wash the slides briefly three times with PBS. Carefully dry around the area to be stained.
7. Cover the sections with a sufficient amount of anti-mouse IgM-fluorescein* solution and incubate in a humid chamber at 37°C for one hour.
8. Wash the slides as described in step 6.
9. Cover the sections with a suitable embedding medium, cover with a cover slip, and examine by fluorescence microscopy.

*HRP or ABC can also be used.

Optimal results can be obtained by titrating the primary and secondary antibodies

Immunocytochemistry

1. Fix the preparations with 4% paraformaldehyde (in PBS) at room temperature for 10 minutes. O4 is a sulfatide which can be dissolved out of the membrane by organic solvents; acetone and methanol should not be used for fixation.
2. Wash the slide three times for 5 min. each in PBS at room temperature.
3. Block the non-specific binding sites by incubating the sections in a human chamber with 5% FCS at room temperature for 30 minutes.
4. Wash the slides as described in step 2.
5. Cover the sections with a sufficient amount of MAB345 (10-20 $\mu\text{g}/\text{mL}$ in PBS) and incubate in a humid chamber at 37°C for one hour.
6. Wash the slides briefly three times with PBS. Carefully dry around the area to be stained.
7. Cover the sections with a sufficient amount of anti-mouse IgM-fluorescein solution and incubate in a humid chamber at 37°C for one hour.
8. Wash the slides as described in step 6.
9. Cover the sections with a suitable embedding medium, cover with a cover slip, and examine by fluorescence microscopy.

Note: Do not allow the preparations to dry out during staining.