

**MOUSE ANTI-GALACTOCEREBROSIDE
MONOCLONAL ANTIBODY**

- CATALOG NUMBER:** MAB342 (formerly Roche Catalog Number 1351621)
- LOT NUMBER:**
- QUANTITY:** 50 µg
- CONCENTRATION:** 1 mg/mL
- SPECIFICITY:** Galactocerebroside (GalC), sulfatide, psychosine and other galactolipids (1,5). Cross-reacts with the sulfatide ester of GalC, but to a 16-fold lesser extent. No cross-reactivity with sphingosine, ceramide, mixed ganglioside or glucocerebroside. Binds specifically with oligodendrocytes and Schwann cells.
- IMMUNOGEN:** Synaptic plasma membranes from bovine hippocampus.
- ISOTYPE:** IgG₃
- APPLICATIONS:** Immunohistochemistry: 0.5-10 µg/mL on formalin fixed frozen sections. Can not be used on paraffin embedded tissue sections since the antigen is denatured during embedding and paraffin removal.
Immunocytochemistry: 0.5-10 µg/mL on 4% paraformaldehyde, acetic acid or ethanol fixed cultured cells.
ELISA with purified galactocerebroside.
Optimal working dilutions must be determined by the end user.
- SPECIES REACTIVITIES:** Human (3), rat (1), rabbit (4), mouse, chicken and bovine.
- FORMAT:** Purified immunoglobulin
- PRESENTATION:** Liquid. Buffer = 10 mM Potassium Phosphate, 150 mM NaCl, pH 7.4 containing 0.09% sodium azide.
- STORAGE/HANDLING:** Maintain frozen at -20°C in undiluted aliquots for up to 6 months after date of receipt. Avoid repeated freeze/thaw cycles.
- REFERENCES:**
- 1) *PNAS.USA* **79**:2709 (1982).
 - 2) *J. Neurosci.* **7**:2936 (1987).
 - 3) *J. Neurochem.* **51**:380 (1988).
 - 4) *J. Compar. Neurol.* **244**:128 (1985).
 - 5) *J. Neurosci. Res.* **24**:548 (1989).
 - 6) *Am. J. Clin. Pathol.* **75**:734 (1981).
 - 7) Patte-Mensah, C., et al., *J. Neurochemistry* (2003) **86**:1233-1246.
 - 8) Sonntag, K., et al., *European Journal of Neuroscience* (2004) **19**:1141-1152.

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APPLICATION NOTES FOR MAB342

Immunohisto/cytochemistry

Anti-GalC can be used to stain fresh or formalin-fixed frozen sections (4). This antibody can not be used on paraffin sections because the procedure of embedding tissue in paraffin will extract the antigen recognized by the GalC monoclonal.

Paraformaldehyde-fixed or living cell cultures [post fixed at -20C with ethanol/acetone, 95:5 (v/v)] can also be stained with anti GalC. The procedure below was designed to evaluate GalC (oligodendrocyte) distribution in fixed neonatal rat cerebral cortex cultures.

- 1) Incubate coverslip cultures in 4% paraformaldehyde (v/v) for ten minutes at room temperature.
- 2) Wash three times with fresh PBS, five minutes per wash.
- 3) Block nonspecific staining by incubating the coverslip cultures in PBS containing 1% normal goat serum (v/v) for 60 minutes at room temperature. (The serum should be from the same host as the animal source for the secondary antibody.)
- 4) Gently dispense 50-100 μ L of the appropriately diluted Anti-GalC solution onto the surface of each coverslip culture. An antibody concentration of 5 μ g/mL works well for most applications. The antibody should be diluted in PBS containing no less than 150 mM NaCl and 1% normal goat serum. Prepare working dilutions each day before use.
- 5) Incubate in a humid chamber for 60 minutes at room temperature.
- 6) Wash three times with PBS at room temperature, five minutes per wash.
- 7) Gently dispense 50-100 μ L of appropriately diluted FITC conjugated goat anti-mouse IgG F(ab')₂ onto the surface of each coverslip. Peroxidase-conjugated goat anti-mouse IgG (F(ab')₂) may also be used.
- 8) Incubate for 60 minutes at room temperature.
- 9) Wash three times with PBS at room temperature, five minutes per wash.
- 10) Place a small drop of aqueous mounting medium on a clean glass microscope slide, and place the coverslip culture, cell-surface-down, onto the drop of mounting medium.

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