

TECHNICAL INSTRUCTIONS

Description	Clone	Species	Ig-isotype	Catalogue No.
Monoclonal antibody to multidrug resistance protein 4 (MRP4, ABCC4)	M ₄ I-10	rat	IgG2a	SB- M ₄ I-10-MAB

Specificity: The M₄I-10 Mab was selected after immunization with a fusion protein containing the *E. coli* maltose binding protein and a fragment of the human MRP4 protein corresponding to amino acids 372-431. MRP4 transports cyclic nucleotides and anti-retroviral compounds. The M₄I-10 Mab also reacts with the mouse orthologue of the transporter molecule (Mrp4).

Applications: Immunoblotting, Immunocytochemistry, Immunohistochemistry and Flow cytometry

Immunoblotting: use 1:20-50 dilution and HRP-anti-rat-IgG.

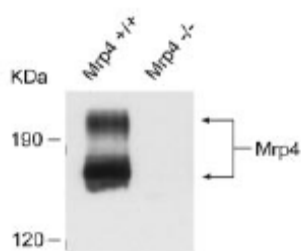


Figure 1. Equal samples of total brain protein (10 µg) from Mrp4^{+/+} and Mrp4^{-/-} animals were fractionated by polyacrylamide gel electrophoresis and treated with the monoclonal antibody M4I-10.

Immunocytochemistry: use 1:20-50 dil. on acetone fixed cytospin preparations.

Immunohistochemistry: M₄I-10 (use 1:20) on acetone fixed frozen sections can be followed by incubation with biotin-labeled rabbit anti-rat IgG (1:100, DAKO) and HRP-labeled streptavidin (1: 500, DAKO)

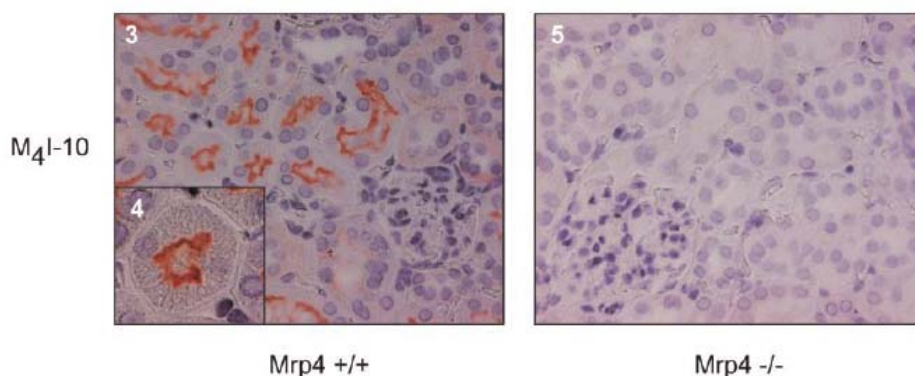


Figure 2. Immunohistochemical analysis of Mrp4 expression in paraffin-embedded kidneys from Mrp4^{+/+} and Mrp4^{-/-} mice. The inset (panel 4) shows the proximal tubules at greater magnification.



Flow cytometry: optimal conditions still to be defined.

Note: Optimal conditions must be determined individually for each application.

Presentation: 1 ml vials (>>200 tests) containing antibody in serumfree culture supernatant, with 0.7% BSA (Roche, Mannheim, Germany) and 0.1% Sodium azide. Concentration 250 µg immunoglobulin/ml (by ELISA)

Shipping: Ambient temperature.

Storage: Maintain refrigerated at 2-8°C for up to 6 months. For long-term storage prepare small aliquots and store at -20°C.

References: Mrp4 confers resistance to topotecan and acts as a double agent in protecting the brain from chemotherapy. Markos Leggas, Masashi Adachi, George L. Scheffer, Daxi Sun, Peter Wielinga, Guoqing Du, Kelly E. Mercer, Yanli Zhuang, John C. Panetta, Brad Johnston, Rik J. Scheper, Clinton F. Stewart, and John D. Schuetz. Mol Cell Biol in press 2004

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LIMITATIONS: This is a laboratory reagent, not to be administered to humans or animals nor used for any drug purpose.

Safety information about the cell lines and culture media used in the production of the MAb.

MAb producing cells: The hybridoma cell line was obtained by fusion of lymph node cells from an immunized rat (Wistar) with SP2/O mouse myeloma cells.

Culture medium: IMDM (BioWhittaker), supplemented with Nutridoma-SP (Boehringer, Indianapolis, USA), without serum or added enzymes. Antibody containing supernatant has been concentrated and filtered through a 0.22 micron filter.

NOTE: this monoclonal antibody has been produced in a clinical laboratory in which no animal viruses are being studied or cultured.

