

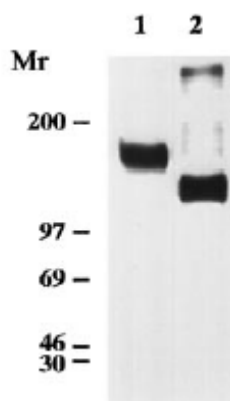
## TECHNICAL INSTRUCTIONS

Description	Clone	Species	Ig-isotype	Catalogue No.
Monoclonal antibody to the Multidrug Resistance-related Protein MRP1	MRPr1	rat	IgG2a	SB- MRPr1-MAB

**Specificity:** MRPr1 reacts with an epitope (amino acids 238-247, Hipfner et al, 1998) of MRP1, a 180-195 kD transmembrane transporter protein overexpressed in various human non-P-glycoprotein MDR tumor cell lines. MRPr1 was raised against a bacterial fusion protein of MRP, containing a segment of 168 amino acids in the amino-proximal half of the protein. MRPr1 does not cross-react with the human MDR1 and MDR3 gene products (Flens et al. 1994), nor with MRP2, 3 and 5 (Scheffer et al, 2000).

**Applications:** Immunoblotting, Immunohistochemistry, Flow cytometry and Immunofluorescence

**Immunoblotting:** Western Blot (Fig. 1 )

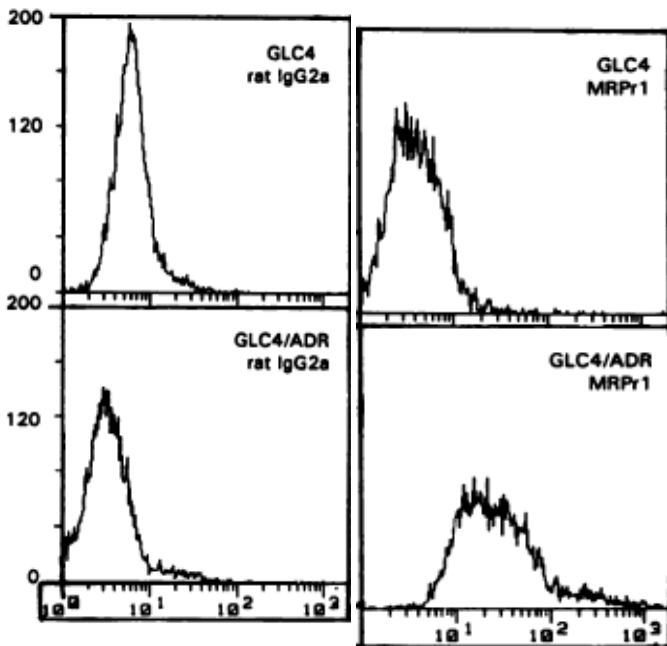


**Figure 1.** Immunoblot analysis of MRP1 by mAb MRPr1. Isolated membranes were subjected to electrophoresis and immunoblotting. The samples were obtained from HL60-MRP1 cells (*lane 1*), from Sf9 cells expressing MRP1 (*lane 2*). In *lane 1*, 10 mg, and in *lanes 2*, 2 mg of protein were analyzed.

**Immunohistochemical staining:** The antibody can be diluted 1:20 for of frozen sections, air-dried or acetone fixed cells, paraffin-embedded tissue sections.

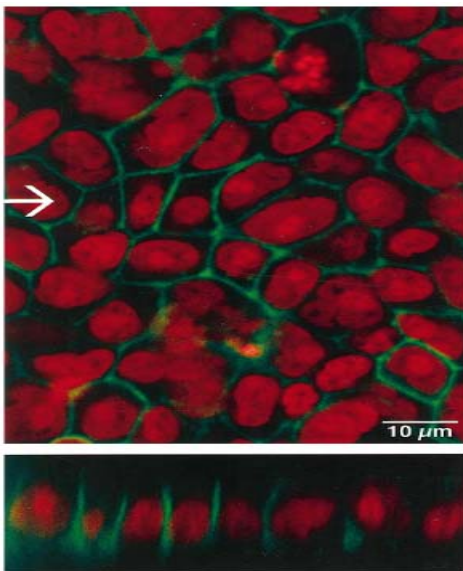


Flow cytometry: cell permeabilization required



**Figure 2.** Flow cytometric analysis of permeabilized small cell lung cancer GLC4 and GLC4/ADR cells detected with MRPr1, and an isotype-matched control rat MAb.

Immunofluorescence with confocal laser scanning microscopy:



**Figure 3.** For immunofluorescence experiments, cells were grown on microporous polycarbonate filters (3-mm pore size, 24.5-mm diameter, (Transwell™ 3414; Costar Corp., Cambridge, MA) at a density of  $2 \times 10^6$  cells/well or on microscope slides for 3 days. No difference was observed between the two procedures. Immunodetection of human MRP1 with monoclonal antibody MRPr1 were performed.

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



**SOLVO**  
BIOTECHNOLOGY

**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:** Flens et al. Cancer Res. 54: 4557, 1994;  
Cole et al. Science 258: 1650, 1992;  
Zaman et al: PNAS 91: 8822-8826, 1994;  
Schadendorf et al. Am J Pathol 147: 1545, 1995;  
Flens et al. Am J Pathol 148: 1237, 1996;  
Evers et al J Clin Invest. 97: 1211, 1996;  
Nooter et al: Ann. Oncol. 7: 75-81, 1996;  
Stein et al. JNCI 89: 807, 1997;  
Den Boer et al. Leukemia 11: 1078, 1997;  
Nooter et al. Br J Cancer 76: 486, 1997.  
Hipfner et al. Br J Cancer 78: 1134, 1998;  
Bakos et al J Biol Chem 273, 32167, 1998;  
Scheffer et al. Cancer Res 60: 5269, 2000

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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.

