

## TECHNICAL INSTRUCTIONS

Description	Clone	Species	Ig-isotype	Catalogue No.
Monoclonal antibody to the Multidrug Resistance-related Protein MRP1	MRPm6	mouse	IgG1	SB- MRPm6-MAB

**Specificity:**

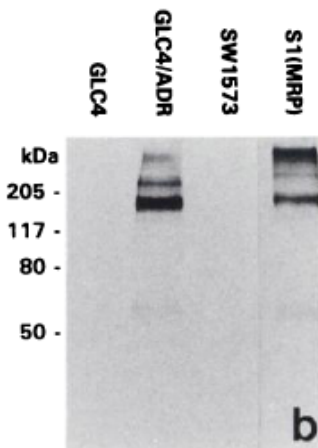
MRPm6 reacts with an internal epitope (amino acids 1511-1520, Hipfner et al, 1998) of MRP1, a 180-195 kD transmembrane transporter protein overexpressed in various human non-P-glycoprotein MDR tumor cell lines. MRPm6 was raised against a bacterial fusion protein of MRP1, containing a segment of 170 amino acids in the carboxy terminal end and part of the carboxy proximal nucleotide binding domain of the protein. MRPm6 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, Immunocytochemistry, Immunohistochemistry and Flow cytometry

Immunoblotting:

use primary antibody in 1:500 dilution.



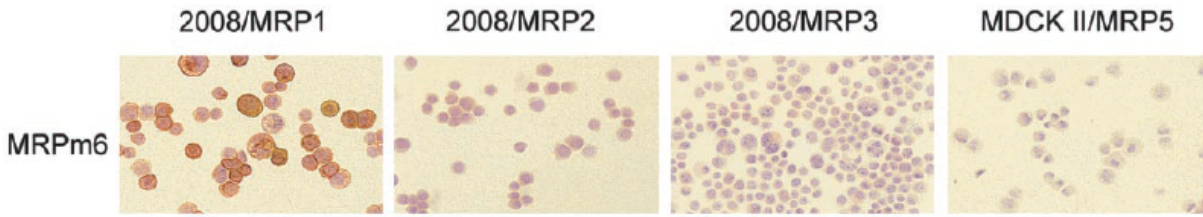
**Figure 1.**

Protein blot of SDS-PAGE of total protein isolates of cell lines with increased levels of MRP [GLC4/ADR and SI (MRP)] and the corresponding parental drug-sensitive cell lines (GLC4 and SW-1573) incubated with MRPm6 Mab.

Immunohistochemical and immunocytochemical staining:

the antibody can be diluted 1:20 for frozen sections, air-dried or acetone fixed cells, paraffin-embedded tissue sections.

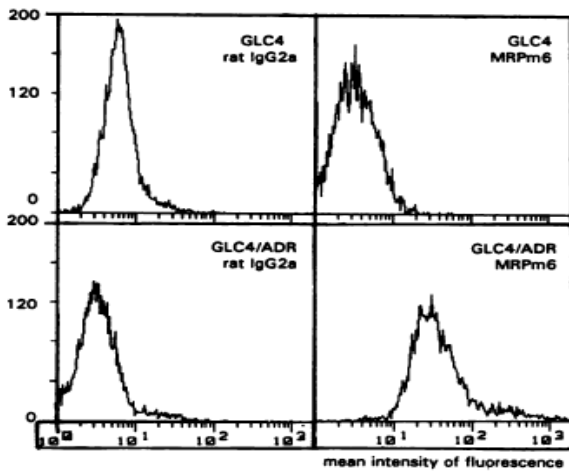




**Figure 2.**

Staining of cytopins of the ovarian 2008 and canine kidney MDCK II cells transfected with MRP1, MRP2, MRP3, or MRP5 cDNA with MRPm6 Mab. Color development was with amino-ethyl-carbazole. Strong staining of MRP1 transporter molecules is observed by MRPm6 Mab.

Flow cytometry: dilute primary antibody in 1:20 (note: cell permeabilization required).



**Figure 3.**

Flow cytometric analysis of permeabilized small cell lung cancer GLC4 and GLC4/ADR cells detected with MRPm6 and an isotype-matched control rat MAb.

*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:** Flens et al Cancer Res. 54: 4557-4564, 1994;  
Schadendorf et al Am J Path 147: 1545, 1995;  
Flens et al Am J Path, 148: 1237, 1996;  
Den Boer et al Leukemia 11: 1078, 1997;  
Hipfner et al Br J Cancer 78: 1134, 1998;  
Bakos 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 mouse (Balb/c) 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.

