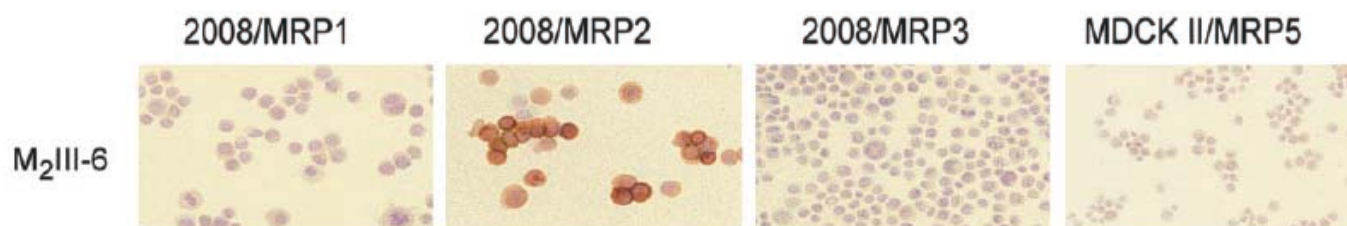


## TECHNICAL INSTRUCTIONS

Description	Clone	Species	Ig-isotype	Catalogue No.
Monoclonal antibody to Multidrug Resistance Protein 2 (MRP2/cMOAT)	M <sub>2</sub> III-6	mouse	IgG2a	SB- M <sub>2</sub> III-6-MAB

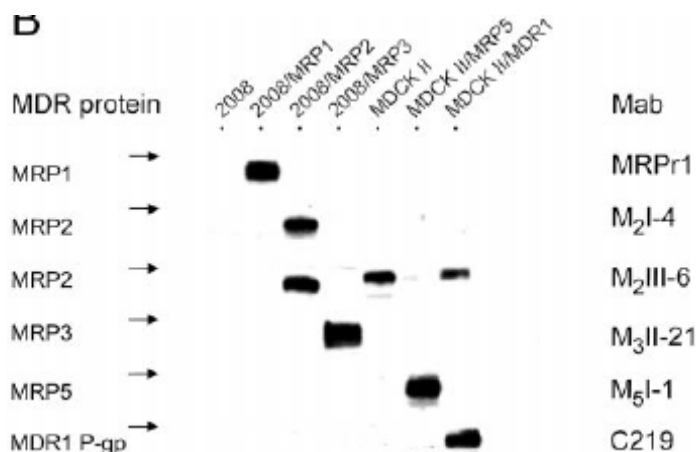
**Specificity:** M<sub>2</sub>III-6 reacts with an internal epitope of MRP2, a 190-200 kD transmembrane protein earlier known as the canalicular multi-organic anion transporter cMOAT, absent in patients with the Dubin-Johnson syndrome, an autosomal recessive liver disorder characterized by chronic conjugated hyperbilirubinemia. MRP2 is a member of the MRP family of multidrug resistance related proteins, and MRP2 overexpression has been observed in a subset of cisplatin resistant cell lines. M<sub>2</sub>III-6 was raised against a fusion protein of the bacterial maltose binding protein and rat Mrp2, containing the 202-amino acid COOH terminal end of the transporter protein. The MAb detects both rat and human MRP2. M<sub>2</sub>III-6 does not cross-react with the human MDR1/P-gp, MRP1, MRP3 or MRP5 gene products. (Fig. 1.)



**Figure 1.** M<sub>2</sub>III-6 did not cross-react with MRP1, MRP3 or MRP5 proteins.

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

**Immunoblotting:** use M<sub>2</sub>III-6 Mab in 1:20-50 dilution, and anti-mouse-HRP in 1:1000 dilution (Dako). (Fig. 2)



**Figure 2.** Western blot with monoclonal antibodies of parental and transfected ovarian 2008 and canine kidney MDCK II cells, probed for MRP1 (Mab: MRPr1), MRP2 (Mabs: M<sub>2</sub>I-4, M<sub>2</sub>III-6), MRP3 (Mab: M<sub>3</sub>II-21), MRP5 (Mab: M<sub>5</sub>I-1) and P-gp (Mab: C219). Arrow: Mr 200.000 marker. Each of the transporters is specifically recognized by its corresponding MAb. The M<sub>2</sub>III-6 MAb also recognises a band in the parental and P-gp transfected MDCK II cell line, presumably endogenous canine Mrp2.



**SOLVO**  
BIOTECHNOLOGY

- Immunocytochemistry: use 1:20-50 dilution on acetone fixed cytospin preparations
- Immunohistochemistry: Frozen section:  
use 1:20 dilution on acetone fixed frozen sections followed by incubation with rabbit anti-mouse IgG (1:25, Dako) and a monoclonal mouse APAAP complex (1:50, Dako)
- Paraffin section:  
after pretreatment with 0.01 M citric acid (pH 6.0) in distilled water at 100 C for 5 min 3 times. After incubation of M2III-6 (use 1:20) and washing, slides can be incubated with biotinylated rabbit anti-mouse IgG (1:100, Jackson, West Grove) and streptavidin conjugated to horseradish peroxidase (1:500, Zymed, San Francisco, CA).
- 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:**
- \*Paulusma et al, Congenital jaundice in rats with a mutation in a multidrug resistance-associated protein gene. Science 271: 1126-1128, 1996.
  - \*Kool et al. Analysis of expression of cMOAT (MRP2), MRP3, MRP4, and MRP5, homologs of the multidrug resistance-associated protein gene (MRP1), in human cancer cell lines. Cancer Res 57: 3537-3547, 1997.
  - \*Scheffer et al. Specific detection of multidrug resistance proteins MRP1, MRP2, MRP3, MRP5 and MDR3 P-glycoprotein with a panel of monoclonal antibodies. 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.

