

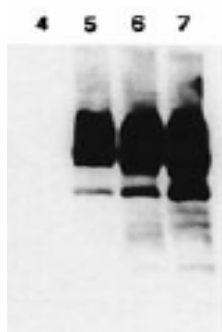
## TECHNICAL INSTRUCTIONS

Description	Clone	Species	Ig-isotype	Catalogue No.
Monoclonal antibody to human MRP3	M <sub>3</sub> II-9	mouse	IgG1	SB- M <sub>3</sub> II-9-MAB

**Specificity:** M<sub>3</sub>II-9 reacts with an internal epitope of MRP3, a 190-200 kD transmembrane protein that is closely related to the multidrug resistance protein MRP1. M<sub>3</sub>II-9 was raised against a bacterial fusion protein of MRP3, containing amino acids 830-949 of the protein. M<sub>3</sub>II-9 does not cross-react with the human MDR1, MRP1, MRP2 or MRP5 gene products.

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

Immunoblotting: use 1:20-50 dilution, and anti-mouse-HRP (Fig. 1)



**Figure 1.**

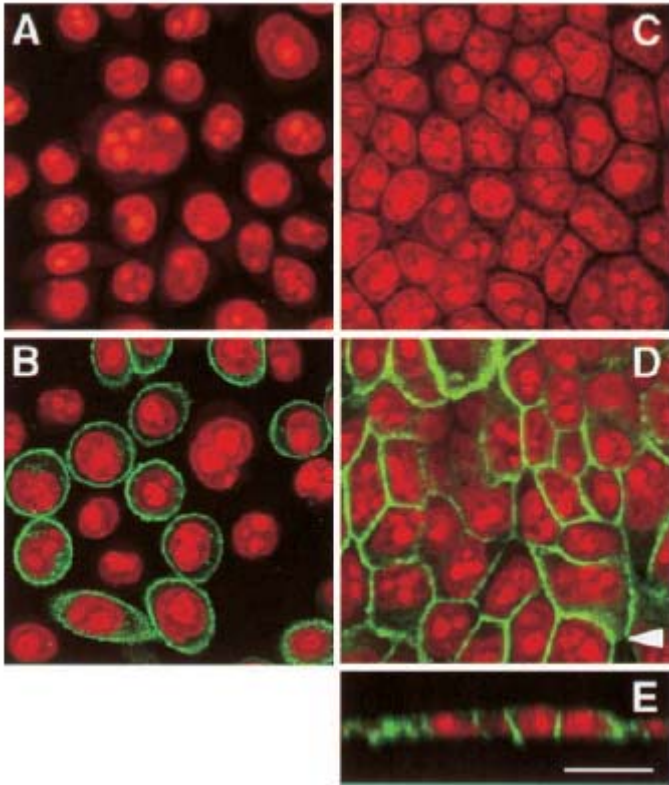
Western blot analysis of MRP3 protein in parental cells and several clones of transduced MDCKII cells. Total cell lysates (5 mg/lane for the MDCKII cells) were size fractionated in a 7.5% polyacrylamide gel containing 0.5% SDS. The fractionated proteins were transferred to a nitrocellulose membrane, and MRP3 was detected by incubation with monoclonal antibody M<sub>3</sub>II-9. 4=MDCKII; 5=MDCKII/MRP3-17; 6=MDCKII/MRP3-18; 7=MDCKII/MRP3-20.

Immunocytochemistry: use 1:20-50 dilution on acetone or formaldehyde fixed cytospin preparations

Immunofluorescence with confocal laser scanning microscopy:

cells were grown overnight on glass slides for 3 days on microporous polycarbonate filters (3-mm pore size, 24.5-mm diameter, Transwell 3414; Costar, Cambridge, MA). Detection of MRP3 in these cells with monoclonal antibody M<sub>3</sub>II-9 as primary antibody and Alexa 488 labeled goat-anti-mouse IgG (1:50, Molecular Probes) as secondary antibody



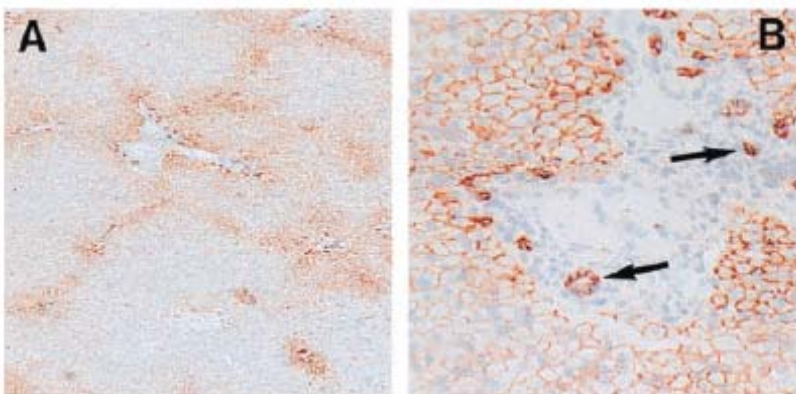


**Figure 2.**

Immunolocalization of MRP3 in 2008 cells and MDCKII monolayers by confocal laser scanning microscopy. MRP3 is detected by indirect immunofluorescence (green signal) with monoclonal antibody M<sub>3</sub>II-9. Nucleic acids were detected by counterstaining with propidium iodide (red signal). (A) Parental 2008 cells; (B) 2008yMRP3-8 cells; (C) parental MDCKII cells; (D) MDCKIIyMRP3-17 cells; (E) vertical XyZ section of the monolayer shown in D. The arrow in D indicates the position where the XyZ section was made. (Bar 5 20 mm.)

Immunohistochemistry:

M<sub>3</sub>II-9 (use 1:20) on acetone fixed frozen sections can be followed by incubation with rabbit anti-mouse IgG (1:25, Dako) and a monoclonal mouse APAAP complex (1:50, Dako). Alternatively, after incubation with M<sub>3</sub>II-9 (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).



**Figure 3.**

Immunohistochemical staining of human liver. (A and B) Immunohistochemical detection of MRP3 in the lateral membranes of cholangiocytes (indicated by arrows) and (baso)lateral membranes of hepatocytes surrounding the portal tracts by using monoclonal antibody M<sub>3</sub>II-9. B is an enlargement of A.

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:** 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.  
Kool et al. MRP3, an organic anion transporter able to transport anti-cancer drugs. *Proc Natl Acad Sci USA* 96: 6914-6919, 1999.  
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.

**Correspondence to:**

Name: **Tünde Nagy, Ph.D.**  
Production Manager

Mail: **SOLVO Biotechnology**  
H-6726 Szeged  
Közep fasor 52.  
Hungary

E-Mail: t.nagy@solvo.com

Phone: +36 62 424 729

Cell: +36 30 676 8640

Fax: +36 62 426 098

**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.

