

TECHNICAL INSTRUCTIONS

Description	Clone	Species	Ig-isotype	Catalogue No.
Monoclonal antibody to MVP (major vault protein, also known as the lung resistance protein, LRP).	LRP-56	rat	IgG2b	SB- LRP-56-MAB

Specificity: LRP-56 reacts with an internal epitope of the LRP/ Major Vault Protein (P110), which is overexpressed in various human non-P-glycoprotein MDR tumor cell lines.

Applications: Immunoblotting, Immunocytochemistry, Immunohistochemistry and Flow cytometry

Immunoblotting: use primary antibody in dilution 1:500.

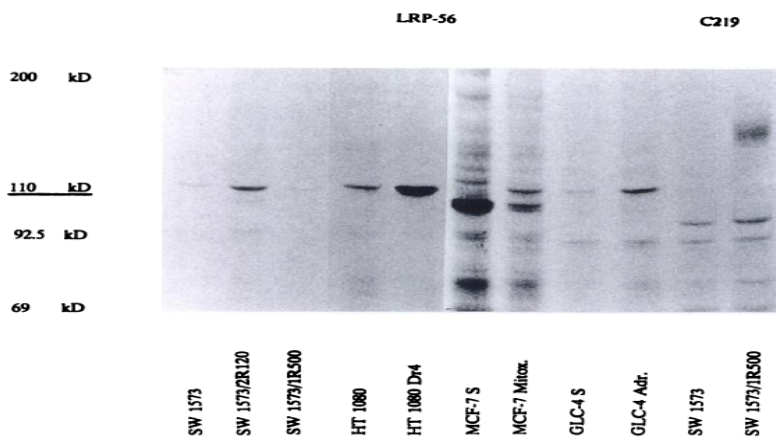


Figure 1.

Immunoprecipitation with LRP-56 of the parental (SW-1573), non-Pgp MDR (2R/120) and Pgp-positive (IR500) MDR cell lines (Lanes 1, 2, and 3). Results for the HT 1080, MCF-7 and GLC-4 cells are shown in Lanes 4, 6 and 8 (parental cell lines) and 5, 7 and 9 (non-Pgp MDR counterparts). Lanes 10 and 11, control runs with C219 precipitates from the SW-1573 and Pgp-positive IR500 cell lines, respectively. No bands were overexpressed when C219 or JSB-1 was used for immunopredpitations in any of the non-Pgp MDR cell lines, kD: molecular weight in thousands.

Immunocytochemical staining: cytopspins were incubated with LRP-56 (1:100 dilution, 60 min) and control antibodies, followed by incubation with rabbit anti-mouse immunoperoxidase (Dako, Copenhagen, Denmark; 1:25, 30 min), and developed with 3,3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO):0.5 mg/ml PBS containing 0.015% H₂O₂.



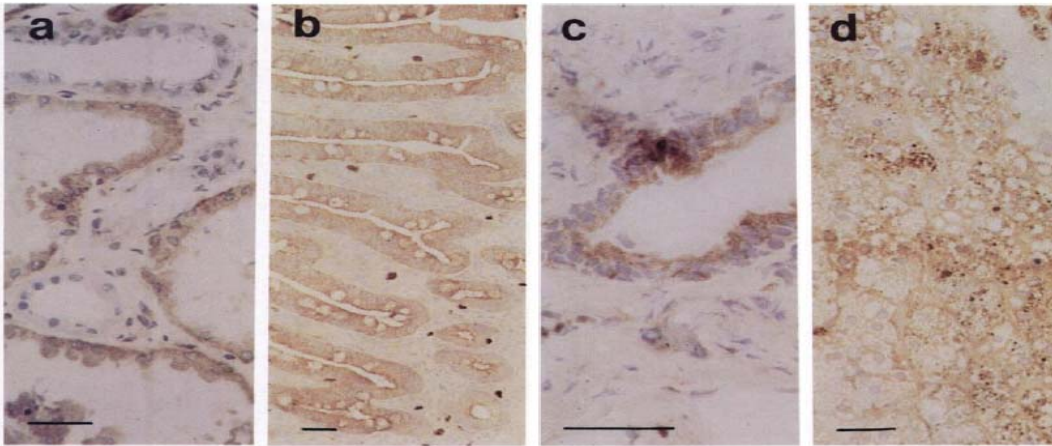


Figure 2.

Immunohistochemical staining: reactivity of paraffin sections of normal human tissues with the LRP-56 monoclonal antibody. a) kidney; b) colon; c) bronchus; d) adrenal.

Immunohistochemical staining:

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

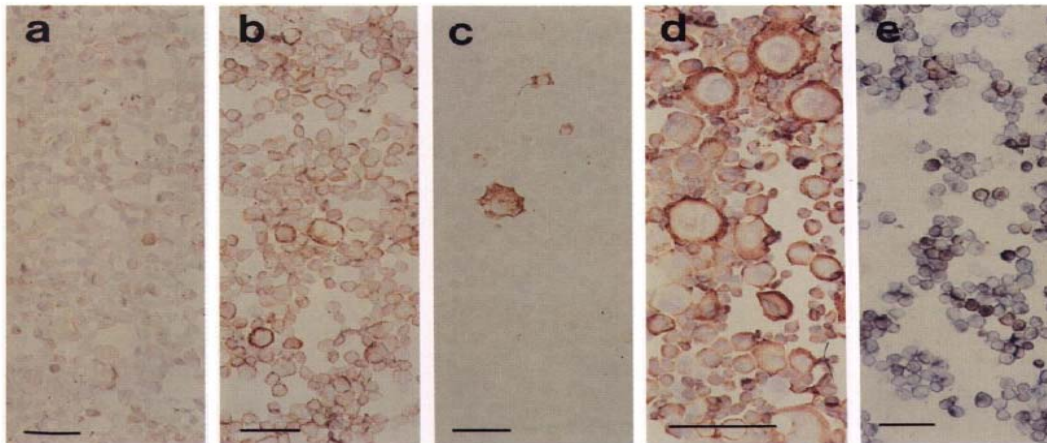


Figure 3.

LRP-56 staining of cytopsin preparations of parental SW-1573 cells (a), 2R120 non-Pgp MDR cells (b, d), 2R160 Pgp-positive cells (c) and MCF7/D40 Pgp-positive MDR breast cancer cells (e). The MCF7/40 MDR tumor cells were counterstained with C219 to show plasma membrane-bound Pgp (blue staining). Bar, 50 μ m.

Flow cytometry: same as for immunohistochemical staining (note: cell permeabilization required).

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 μ g 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.



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References: Scheper RJ et al., Cancer Res. 53: 1475, 1993;
Scheffer GL et al, Nature. 1: 578, 1995;
Izquierdo MA et al., JNCI 87: 1230, 1995;
Schadendorf D et al., Am J Path. 147: 1545, 1995;
List AF et al. Blood 87: 2464, 1996;
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Stein et al. PNAS 89:807, 1997;
Den Boer et al, Leukemia 11: 1078, 1997;
Schroeijers et al, Am J Path. 152:373, 1998;
Kickhoefer VA et al., J. Biol. Chem. 273: 8971, 1998;
Kickhoefer VA et al., J. Cell Biol. 146: 917, 1999;
Schroeijers AB et al., Cancer Res. 60: 1104, 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.

