

TECHNICAL INSTRUCTIONS

Description	Clone	Species	Ig-isotype	Catalogue No.
Monoclonal antibody to Breast Cancer Resistance Protein (BCRP, ABCG2)	BXP-34	mouse	IgG1	SB- BXP-34-MAB

Specificity: BXP-34 Mab was selected after immunization with the mitoxanthrone resistant, BCRP overexpressing cell line MCF7 MR. BXP-34 reacts with an internal epitope of BCRP, a 70 kD transmembrane half-transporter, which is involved in multidrug resistance. BXP-34 did not cross-react with the human MDR1, MRP1, MRP2, MRP5 gene products.

Applications: Immunoblotting, Immunocytochemistry, Immunohistochemistry and Flow cytometry

Immunoblotting: the Mab is unreactive.

Immunocytochemistry: use 1:20-50 dilution on acetone fixed cytopsin preparations.

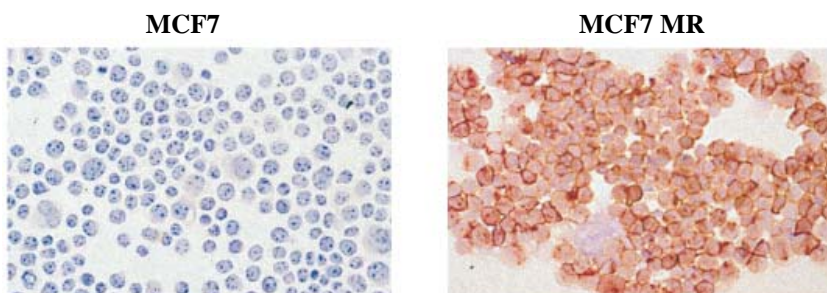


Figure 1. Immunostaining of cytopsin of parental and drug-selected sublines of MCF-7 breast cancer cells with the BXP-34 Mab. The MR sublines are MTX-selected.

Immunohistochemistry: BXP-34 (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). BXP-34 cannot be used on formaldehyde-fixed paraffin-embedded human tissues and tumours.

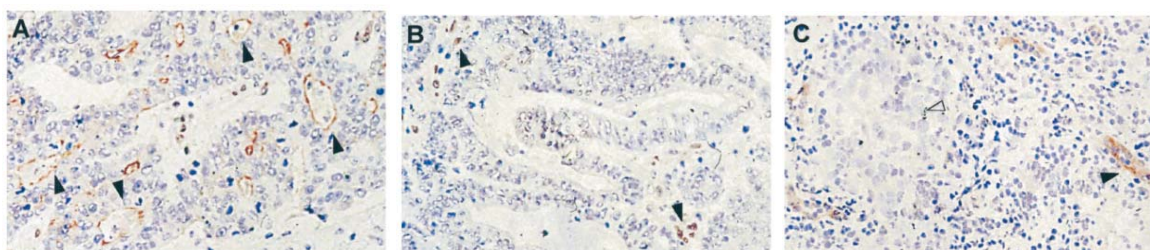


Figure 2. Immunohistochemical staining with the BXP-34 Mab, of two small-intestine tumors (A, B) and a testicular tumor (C). Biotinylated rabbit-antimouse serum and HRP-labeled streptavidin were used as secondary reagents. Color development was with AEC. A, BCRP staining of the epithelial cells of the neoplastic crypts. B, no staining is observed, except for some desmoplastic stroma staining. C, the seminoma cells are negative, but staining of the endothelial cells is observed.



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:

1. JW Jonker, M Buitelaar, E Wagenaar, MA van der Valk, GL Scheffer, RJ Scheper, T Plösch, F Kuipers, RPJ Oude Elferink, H Rosing, J Beijnen, AH Schinkel. The breast cancer resistance protein protects against a major chlorophyll-derived dietary phototoxin and protoporphyria. PNAS in press 2002.
2. Doyle LA, Yang WD, Abruzzo LV, Krogmann T, Gao YM, Rishi AK, and Ross DD. A multidrug resistance transporter from human MCF-7 breast cancer cells [erratum in PNAS USA 1999; 96(5):2569]. Proc Natl Acad Sci USA 95: 15665-15670, 1998.
3. Scheffer GL, Maliepaard M, Pijnenborg ACLM, van Gastelen M A, de Jong MC, Schroeijers AB, van der Kolk DM, Allen JD, Ross DD, van der Valk P, Dalton WS, Schellens JHM and Scheper RJ. Breast Cancer Resistance Protein is localized at the plasma membrane in mitoxantrone and topotecan resistant cell lines. Cancer Res, 60: 2589-2593, 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.

