

TECHNICAL INSTRUCTIONS

Description	Clone	Species	Ig-isotype	Catalogue No.
Monoclonal antibody to Breast Cancer Resistance Protein (BCRP, ABCG2)	BXP-21	mouse	IgG2a	SB-BXP21-MAB

Specificity: BXP-21 MAb was selected after immunization with a fusion protein consisting of the *Escherichia coli* maltose-binding protein and a 126 amino acids part of the BCRP peptide (aa 271-396 of BCRP (GenBank accession no. AF098951)). BXP-21 reacts with an internal epitope of BCRP, a 70 kD transmembrane half-transporter, which is involved in Multidrug resistance. BXP-21 did not cross-react with the human *MDR1*, *MRP1*, *MRP2* gene products (Fig.1).

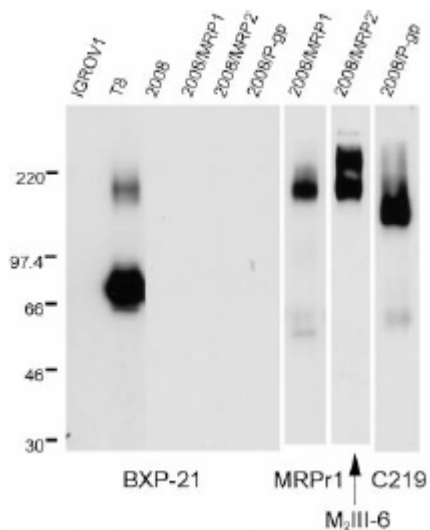


Figure 1. Western blot analysis of BCRP, MRP1, MRP2 and P-gp in total cell lysates from parental and BCRP overexpressing T8 tumor cells as well as MRP1, MRP2 and P-gp transfected cell lines. Blots were hybridized using BXP-21, MRPr1, M2III-6 and C219 primary antibodies. BXP-21 did not crossreact with MRP1, MRP2 or P-gp proteins.

Applications: Immunoblotting, Immunocytochemistry, Immunohistochemistry and Flow cytometry

Immunoblotting: BXP-21 can be used in standard Western blot procedures (Fig. 2)



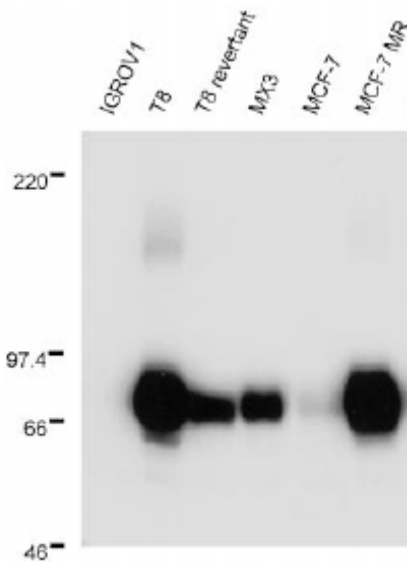


Figure 2.

Western blot analysis of BCRP in total cell lysates from parental (IGROV1, MCF-7) and BCRP overexpressing (T8, T8 revertant, MX3, MCF-7 MR) tumor cell lines. Proteins were hybridized using BXP-21 (1:50) and HRP-conjugated goat antimouse IgG (1:1000; Dako).

Immunocytochemistry: use 1:20-50 dilution on acetone fixed cytopsin 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:
use on formaldehyde-fixed paraffin-embedded human tissues and tumors after pretreated with 10 mM citric acid pH 6.0 for 20 min

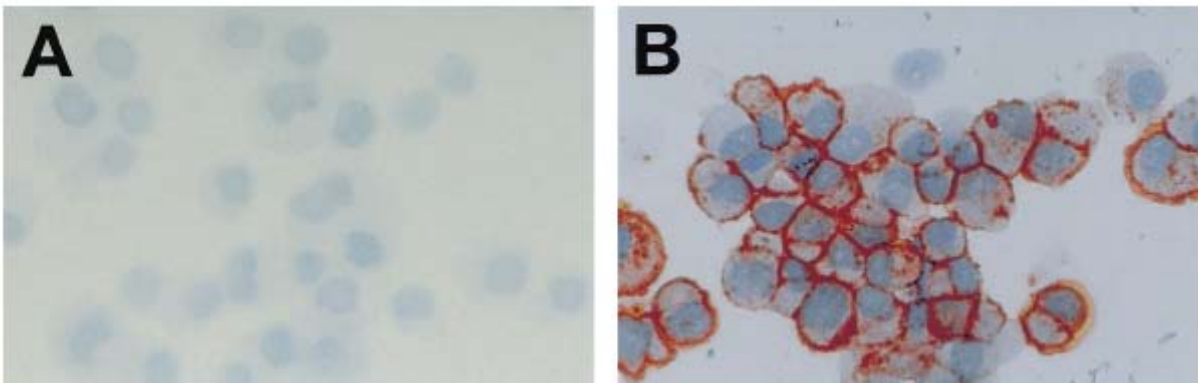


Figure 3.

Immunohistochemistry staining of the parental IGROV1 ovarian tumor cell line and the drug-selected BCRP overexpressing subclone T8 with BXP-21 monoclonal antibody.

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. Doyle, L. A., Yang, W. D., Abruzzo, L. V., Krogmann, T., Gao, Y. M., Rishi, A. K., and Ross, D. D. A multidrug resistance transporter from human MCF-7 breast cancer cells [erratum in PNAS USA 1999; 96(5):2569]. Proc.Natl.Acad.Sci.U.S.A., 95: 15665-15670, 1998.
 2. Scheffer, G. L., Maliepaard, M., Pijnenborg, A. C. L. M., van Gastelen, M. A., de Jong, M. C., Schroeijers, A. B., van der Kolk, D. M., Allen, J. D., Ross, D. D., van der Valk, P., Dalton, W. S., Schellens, J. H. M., and Scheper, R. J. Breast Cancer Resistance Protein is localized at the plasma membrane in mitoxantrone and topotecan resistant cell lines. Cancer Res., 60: 2589-2593, 2000.
 3. Maliepaard M, Scheffer GL, Faneyte IF, van Gastelen MA, Pijnenborg AC, Schinkel AH, van De Vijver MJ, Scheper RJ, Schellens JH. Subcellular localization and distribution of the breast cancer resistance protein transporter in normal human tissues. Cancer Res 2001 Apr 15;61(8):3458-64

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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.

