

TECHNICAL INSTRUCTIONS

Description	Clone	Species	Ig-isotype	Catalogue No.
Monoclonal antibody to the minor vault protein of ~193 kDa, also known as p193 or VPARP.	p193-10	mouse	IgG2a	SB-p193-10-MAB

Specificity: p193-10 reacts with an internal epitope (amino acids 506-510, VALGK) of the minor vault protein (p193 or VPARP), which is overexpressed in various human non-P-glycoprotein MDR tumor cell lines, accordingly to an increase in the number of vault particles. p193-10 was raised against an E. coli lysate transformed with the pET28a(+) expression vector containing amino acids 408-611 of the p193 cDNA.

Applications: Immunoblotting, and Immunohistochemistry

Immunoblotting: the antibody can be diluted 1:10-20 (preferably with ECL).

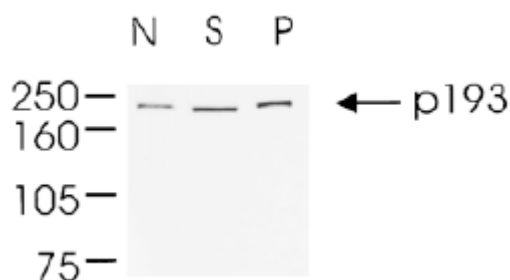


Figure 1.

p193 exists in cells associated with vaults and in other subcellular fractions. Comparison of levels of p193 during biochemical fractionation of HeLa cells. Cellular protein fractions, nuclear (N), supernatant S100 (S), and microsomal pellet P100 (P) were generated from HeLa extracts and resolved by SDS-PAGE. Immunoblot analysis was carried out using affinity-purified polyclonal antibody prepared against recombinant p193 fragment.

Immunohistochemical staining:

the antibody can be diluted 1:10-20 for staining of paraformaldehyde (4%) fixed cytospin preparations and frozen tissue sections. For immunohistochemistry apply the following pretreatment: 10 min 20 mM glycine in PBS (pH 7.5) and 10 min 6 N guanidine hydrochloride in 50 mM Tris-HCl (pH 7.5); Schroeijers et al., 2000.

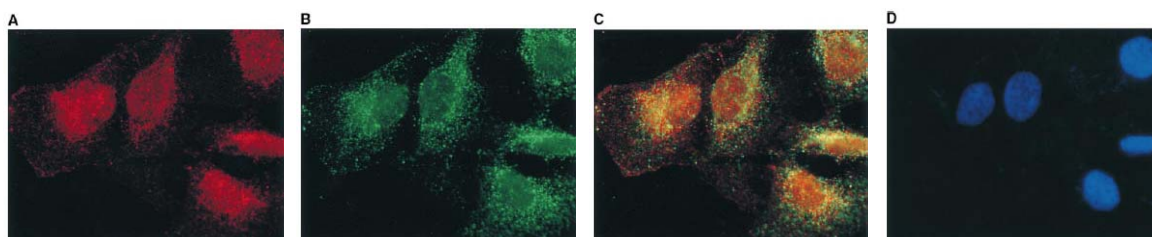




Figure 2.

Subcellular localization of p193. Indirect immunofluorescence of endogenous p193 (A) and vaults (B) in HeLa cells reveals a punctate cytoplasmic staining pattern with some nuclear speckle staining with affinity-purified p193 antibody but not with the vault mAb (LRP56). By merging the images of A and B, coincident staining is seen as yellow (C), revealing a partial overlap in the cytoplasm and highlighting the nuclear staining by p193. The nucleus is stained with DAPI (D). Preimmune p193 antiserum reveals background staining (E). COS cells transiently expressing VSVG-tagged p193 revealed that the recombinant protein has an expression pattern similar to endogenous p193 (F). PARP is predominantly localized to the nucleus (G).

FACS analyses have not yet been 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: Schroeijers AB et al., Cancer Res. 60: 1104, 2000;
Kickhoefer VA et al., J. Cell Biol. 146: 917, 1999;
Kickhoefer VA et al., J. Bio. Chem. 273: 8971, 1998;
Scheper RJ et al., Cancer Res. 53: 1475, 1993;
Scheffer GL et al, Nature Med. 1: 578, 1995;
Izquierdo MA et al., Cytotechnology 27: 137, 1998.

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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.

