BM2311

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Monoclonal Antibody to Melanoma Associated Antigen 450 / 250kDa - Purified

Catalog No.:	BM2311
Quantity:	1 ml
Concentration:	0.1 mg/ml
Host / Isotype:	Mouse / IgG1
Recommended Isotype Controls:	SM10P (for use in human samples), AM03095PU-N
Clone:	NKI/M6
Format:	**State:** Liquid purified Ig fraction. **Buffer System:** PBS with 0.1% BSA as stabilizer and 0.09% Sodium Azide as preservative.
Applications:	Suitable for use in staining of Melanoma Cells in frozen sections by regular immunoperoxidase and Immunofluorescence tests. A 1:5-1:10 dilution in phosphate buffered saline is suggested. For further instruction, see A and I in ''Protocols''. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Specificity:	Recognizes a high molecular weight proteoglycan with a molecular weight of › 450 kD (chondroitin sulfate) and 250 kD (core protein). Reacts strongly with Melanoma Cells derived from cell lines and short term cultures and reacts preferentially with Melanoma Cells in frozen tissue sections. (1) Reacts with most nevi and perineurium and shows weak reactivity with hair follicles.
Storage:	Store the antibody undiluted at 2-8°C for one month or (in aliquots) at -20°C for longer. Avoid repeated freezing and thawing. Shelf life: one year from despatch.
General Readings:	1. de Vries, J.E., et. al., 1986, Int. J. Cancer, 38, 465. 2. Natali P.G., J. Nat'l. Cancer Inst. 67, 591. 3. Buraggi, G.L., et al., 1985, Cancer Res., 45, 3378.
Protocols:	INSTRUCTIONS FOR USE - A Membrane Immunofluorescence: 1. Transfer 5x10e5 cell in 25 µl to a 10x75 mm test tube for staining. 2. Add 25-50 µl of the diluted monoclonal antibody, mix and incubate for 30 minutes on ice. 3. Wash twice with PBS containing 1% BSA and 0.02% Sodium Azide. 4. Add 50 µl of appropriate FITC/TRITC labeled anti mouse Ig antibody, mix and incubate for 30 minutes on ice.

5. Wash twice with PBS containing 1% BSA and 0.02% Sodium Azide.
6. Resuspend the cells in 100 µl washing buffer and analyze on a fluorescence activated cell sorter (FACS) or mount in 90% glycerol, 10% 1 M Tris/HC1, pH 8.0 and analyze by fluorescence microscopy.

INSTRUCTIONS FOR USE - I

Immunoperoxidase Test On Sections:
1. Frozen sections should have been fixed in acetone for 10 min.
2. Incubation in antisera 40-60 min.
3. Incubation in conjugate (e.g. peroxidase conjugated anti mouse IgG), 30 min.
4. Wash in PBS 2 X 5 min.
5. Incubation in AEC, 0.0.1% H2O2, 10 min.
Preparation substrate: 5 mg AEC (3-amino-9-ethylcarbozole) is solubilized in 0.5 ml DMF (dimethylformamide). A glass (or acetone resistant plastic) tube or pipet should be used!
add 9.5 ml. 0.05M NaAc buffer, pH 4.9.
add 5 µl 30% H2O2.

6. Wash in demi water, 2 x 5 min.
7. Slightly counterstain in hematoxylin e.g. 10 sec.
8. Wash in tap water until sections are blue.
9. Mount in aquamont and examin.