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Monoclonal Antibody to Melanoma Associated Antigen (100-7kDa) - Aff - Purified

Catalog No.: BM2313

Quantity: 1 ml

Concentration: 0.1 mg/ml (OD280nm, E1% = 14)

Host / Isotype: Mouse / IgG2b

Clone: NKI/beteb

Format:
State: Liquid
Purification: Protein G affinity purified Ig fraction
Buffer System: PBS, pH 7.4, with 0.02% NaN3 as preservative and 0.1 % BSA as stabilizer.

Applications: Can be used for immunoperoxidase staining on frozen tissue sections. Formalin fixation and embedding in paraffin may affect the reactivity of the antibody. Has not been tested in Western blot. For research studies on frozen or formalin-fixed paraffin embedded tissue sections or on cells grown on coverslips, dilute the antibody 1:5-1:20, preferably in phosphate buffered saline. For further protocols, see instruction for use F, G, H, and I. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.

Specificity: Recognizes a (pre)melanosomal 100 + 7 kd antigen (glycoprotein). Reacts with melanomas, clear cell sarcomas (melanoma of soft tissue), nevocellular nevi, and normal melanocytes. Except for one case of non-Hodgkin's lymphoma in which macrophages were positive, no reactions with other tumors or tissues have been observed.

Storage: Upon receipt, aliquot and store a -20°C. Avoid repeated freezing and thawing. Shelf life: one year from despatch.

General Readings: Vennegoor C. et al., (1988), Am. J. Pathol., 130, 179.

Protocols: INSTRUCTIONS FOR USE-F

METHODS FOR CELLS ON COVERSLIPS:

Indirect Immunofluorescence Microscopy.

1. The cells grown on coverslips are gently washed with PBS at room temperature.
2. Add cold (-/-20 C) methanol and transfer to 2-8°C for 5 minutes.
3. Transfer the coverslips into cold (-/-20 C) acetone in an acetone resistant dish for 2 minutes.
4. Transfer to PBS, rinse a few times.
5. Incubate the coverslips with 100ul monoclonal antibody diluted in PBS for 60 minutes at 37°C.

6. Wash with PBS 3 x 10 minutes.
7. Incubate with FITC or TRITC labeled second antibody directed against mouse IgG at appropriated dilution.
8. Rinse 3 x 10 minutes.
9. Mount in 90% glycerol 10% 1M Tris/HCl pH 8.0.

N.B. Fixation of the cells with formaldehyde or glutaraldehyde is not advisable because of possible destruction of structure and antigenicity. Surveys of fixation procedures used for immunofluorescence microscopy have been given in a number of recent reviews (Fujiwara and Pollard, Osborn and Weber, Lazarides, Wang et al).

In order to reduce background staining dilution of the antibody with PBS supplemented with 0.5% BSA and 0.2% gelatin is recommendable.

Indirect Immunoperoxidase Staining

1. The cells grown on coverslips are gently washed with PBS at room temperature.
2. Add cold (-/-20 C) methanol and transfer to 2-8°C for 5 minutes.
3. Transfer the coverslips into cold (-/-20 C) acetone in an acetone resistant dish for 2 minutes.
4. Transfer to PBS, rinse a few times.
5. Incubate the coverslips with 100ul monoclonal antibody diluted in PBS for 60 minutes at 37°C.
6. Wash with PBS 3 x 10 minutes.
7. Incubate with peroxidase labeled second antibody, 30 - 60 minutes at 37°C.
8. Wash with PBS 3 x 10 minutes.
9. Stain with diaminobenzidin (DAB) solution (0.05% DAB, 50 mm Tris/HCl pH 7.4, 0.01% H202 fresh prepared) during 10 minutes at room temperature.
10. Wash with running tap water, 3 minutes.
11. Counterstain with Mayer's hematoxylin or OsO4.

Fujiwara, K. and Pollard T.D., 1980, in "Current topics in Developmental Biology" Vol. 14, 271-295, Academic Press.Osborn, M and Weber K., 1982, in "Methods in Cell Biology" 24; 97-132, Academic Press.
Lazarides E., 1982, in "Methods in Cell Biology" 24; 313-331, Academic Press.Wang K., Ash, J and Feramisco, 1982, Methods in Enzymology.

INSTRUCTIONS FOR USE-G

Indirect Immunoperoxidase Staining On Frozen Sections

1. 4 to 6 micron thick sections should be used.
2. Sections are thawed, 1-2 hours at room temperature.
3. Tissue is fixed in acetone, 10 minutes.
4. Wash with PBS, 2 x 3 minutes.
5. Incubate with monoclonal antibody (diluted in PBS), 1-2 hours at room temperature.
6. Wash with PBS, 3 x 3 minutes.
7. Incubate with peroxidase labeled second antibody, 30-60 minutes at room temperature.
8. Wash with PBS, 3 x 3 minutes.
9. Stain with diaminobenzidin (DAB) solution 10 minutes at room temperature.
10. Wash with running tap water, 3 minutes.
11. Counterstain with Mayer's hematoxylin, 2 minutes.
12. Wash with running tap water, 5 minutes.

13. Dehydrate with increasing solution of ethanol; 50%, 70%, 96%, absolute, 3 minutes each.
14. Clear with xylol, 3 x 3 minutes.
15. Mount with mounting medium (e.g. malinol).

INSTRUCTIONS FOR USE-H

Indirect Immunoperoxidase Staining On Formalin-Fixed And Paraffin Embedded Tissues

1. 4 micron thick sections should be used.
2. Dewax in xylol, 3 x 3 minutes.
3. Rehydrate in decreasing grades of ethanol:absolute, 96%, 70%, 50%, 3 minutes each.
4. Block endogenous peroxidase activity with freshly made 0.3% H2O2 in methanol, 20 minutes.
5. Wash with PBS, 3 x 3 minutes.
Only if trypinsination is required
5a. Incubate sections with 0.1% Trypsin in 0.1% CaCl2 pH 7.6 for 10 minutes at room temperature.
5b. Wash with PBS, 3 x 3 minutes.
6. Cover the sections with 20% normal rabbit serum in PBS or normal human serum and incubate overnight in a humidity chamber at room temperature to reduce non specific background staining.
7. Decant 20% normal rabbit serum.
8. Incubate with monoclonal antibody (diluted in PBS), 1-2 hours at room temperature.
9. Wash with PBS, 3 x 3 minutes.
10. Incubate with peroxidase labeled second antibody, 30-60 minutes at room temperature.
11. Wash with PBS, 3 x 3 minutes.
12. Stain with diaminobensidin (DAB) solution, 10 minutes at room temperature. A stock solution of 0.5% DAB in 0.5 DAB in 0.5M Tris/HCl (pH7.4) can be made and stored frozen in the dark. Before use a quantity needed for staining can be thawed and diluted 10x with water. The diluted DAB solution should be filtrated. Just before use H2O2 must be added to a final concentration of 0.01%.
13. Wash with running tap water, 3 minutes.
14. Counterstain with Mayer's hematoxylin, 2 minutes.
15. Wash with running tap water, 2 minutes.
16. Dehydrate with increasing solutions of ethanol:50%, 70%, 96%, absolute, 3 minutes each.
17. Clear with xylol, 3 x 3 minutes.
18. Mount with mounting medium (e.g. malinol).

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:
a. 5mg AEC (3-amino-9-ethlcarbozole) is solubilized in 0.5ml DMF (di-methyl-formamide). A glass (or acetone resistant plastic) tube or pipet should be used!
b. add 9.5 ml. 0.05M NaAc buffer, pJ 4.9.
c. add 5ul 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