Melanoma Cocktail

Concentrated and Prediluted Monoclonal Antibody
901-078-051519

BIOCARE MEDICAL

Catalog Number:	**CM 078 B, C**	**PM 078 AA**	**VP 078 G**	**VLTM 078 G20**
Description:	0.5, 1.0 mL, conc.	6.0 mL, RTU	6.0 mL, RTU	20 mL, RTU
Dilution:	1:100	Ready-to-use	Ready-to-use	Ready-to-use
Diluent:	Van Gogh Yellow	N/A	N/A	N/A

Intended Use:
For In Vitro Diagnostic Use
Melanoma Cocktail [HMB45 + M2-7C10 + M2-9E3] is a mouse monoclonal antibody cocktail that is intended for laboratory use in the qualitative identification of HMB45 and MART-1 proteins by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:
The HMB45 clone reacts with a neuraminidase-sensitive oligosaccharide side chain of a glycoconjugate present in immature melanosomes. The HMB45-reactive antigen is present in cutaneous melanocytes, prenatal and infantile retinal pigment epithelium and melanoma cells. It is also thought to be oncofetal in nature. This antibody has been shown to label the majority of melanomas. The MART-1/Melan A recognizes a protein of 18kDa, identified at MART-1 (Melanoma Antigen Recognized by T cells 1) or Melan-A. The MART-1 recognizes a subcellular fraction found in melanosomes. The antibody labels melanomas and tumors showing melanocytic differentiation (1,2). It does not mark neoplasms of epithelial origin, lymphomas or mesenchymal tumors. Melan-A is a useful addition to melanoma panels which are specific to melanocytic lesions. Studies have also shown that MART-1 is more sensitive than HMB45 when labeling metastatic melanomas. HMB45 and MART-1 are coexpressed in the majority of melanomas, as well as solely expressed in certain cases. Thus, the HMB45 and MART-1 cocktail is potentially more sensitive than HMB45 and MART-1 alone. The MART-1 is a cocktail of clones M2-7C10 + M2- 9E3. The combination of HMB45 and the MART-1 cocktail make this triple antibody cocktail a first-order pan melanoma screener (3).

Principle of Procedure:
Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Source: Mouse monoclonal
Species Reactivity: Human; others not tested
Clone: HMB45 + M2-7C10 + M2-9E3
Isotype: IgG1/kappa + IgG2b + IgG2b
Protein Concentration: Call for lot specific IgG concentration.
Epitope/Antigen: HMB45 + MART-1
Cellular Localization: Cytoplasmic
Positive Tissue Control: Metastatic melanoma in lymph node
Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative
Storage and Stability:
Store at 2ºC to 8ºC. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Protocol Recommendations (VALENT® Automated Slide Staining Platform):
VLTM078 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:
- DAB Chromogen Staining Option:
Deparaffinization: Deparaffinize for 8 minutes with Val DePar.
Pretreatment: Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X – OR – Val AR-Lo pH, 5X (use at 1X).
Peroxidase Block: Block for 5 minutes with Val Peroxidase Block.
Protein Block (Optional): Incubate for 10-20 minutes with Val Background Block.
Primary Antibody: Incubate for 30 minutes.
Secondary: Incubate for 10 minutes with Val Mouse Secondary.
Linker: Incubate for 10 minutes with Val Universal Linker.
Polymer: Incubate for 10 minutes with Val Universal Polymer.
Chromogen: Incubate for 5 minutes with Val DAB.
Counterstain: Counterstain for 5 minutes with Val Hematoxylin.
- Red Chromogen Staining Option:
Deparaffinization: Deparaffinize for 8 minutes with Val DePar.
Pretreatment: Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X – OR – Val AR-Lo pH, 5X (use at 1X).
Protein Block (Optional): Incubate for 10-20 minutes with Val Background Block.
Primary Antibody: Incubate for 30 minutes.
Polymer: Incubate for 45 min with Val Mouse AP Polymer.
Chromogen: Incubate for 15 min with Val Fast Red.
Counterstain: Counterstain for 5 minutes with Val Hematoxylin.

Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 1.
Pretreatment: Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.
Protein Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.
Primary Antibody: Incubate for 30 minutes at RT.
Probe: Incubate for 10 minutes at RT with a secondary probe.
Polymer: Incubate for 10-20 minutes at RT with a tertiary polymer.
Chromogen: Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Warp Red.
Counterstain:
Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.
Technical Note:
This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

Rev: 062117

EC REP EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080

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Protocol Recommendations (Ventana BenchMark XT / ULTRA):
VP078 is intended for use with the BenchMark XT / ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

- Using ***ultra*View on XT / ULTRA:**

Template/Detection: ultraView DAB
Pretreatment Protocol: ULTRA CC1 Standard
Primary Antibody: 32 minutes, 37°C

- Using **OptiView on ULTRA:**

Template/Detection: OptiView DAB IHC
Pretreatment Protocol: CC1 32 minutes
Peroxidase: Pre Primary Peroxidase Inhibitor
Primary Antibody: 16 minutes, 36°C

Limitations:
The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

Quality Control:
Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

Precautions:
1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN_3) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (7)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (8)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at http://biocare.net.

Troubleshooting:
Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

References:

1. Blessing K, Sanders DS, Grant JJ. Comparison of immunohistochemical staining of the novel antibody melan-A with S100 protein and HMB-45 in malignant melanoma and melanoma variants. Histopathology. 1998 Feb; 32(2):139-46.
2. Jungbluth AA, *et al*. A103: An anti-melan-a monoclonal antibody for the detection of malignant melanoma in paraffin-embedded tissues. Am J Surg Pathol. 1998 May;22 (5):595-602.

References Cont'd:

3. Beaty MW, *et al*. Effusion cytology of malignant melanoma. A morphologic and immunocytochemical analysis including application of the MART-1 antibody. Cancer. 1997 Feb 25;81(1):57-63.
4. Bonetti F, *et al*. False-positive immunostaining of normal epithelia and carcinomas with ascites fluid preparations of antimelanoma monoclonal antibody HMB45. Amer J Clin Pathol. 1991 Apr;95(4):454- 9.
5. Leong AS-Y, Millos J. An assessment of a melanoma-specific antibody (HMB-45) and other immunohistochmical markers of malignant melanoma in paraffin-embedded tissues. Surg Pathol. 1989;2:137.
6. Ordonez NG, *et al.* Comparison of HMB-45 monoclonal antibody and S-100 protein in the immunohistochemical diagnosis of melanoma. Amer J Clin Pathol. 1988 Oct;90(4):385-90.
7. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
8. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

EC REP EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

IVD CE
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