BIOCARE MEDICAL

ISO 9001&13485 CERTIFIED

Melanoma Cocktail

Prediluted Cocktail Antibody

Control Number: 901-078VP-020316

VP Echelon™ Series

Catalog Number: **VP 078 G**

Description: 6.0 ml, prediluted

Dilution: Ready-to-use

Intended Use:

For In Vitro Diagnostic Use

Melanoma Cocktail [HMB45 + M2-7C10 + M2-9E3] is 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. 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, an enzyme labeled polymer is added to bind to the primary antibody. The detection of the bound antibody is 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

Total Protein Concentration: ~10 mg/ml. Call for lot specific IgG concentration.

Epitope/Antigen: HMB45 and MART-1

Cellular Localization: Cytoplasmic

Positive 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. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user.

Protocol Recommendations:

Using ***ultra*VIEW™ Detection Kit**

Pretreatment Solution (recommended): CC1

Pretreatment Protocol: Standard

Primary Antibody: Incubate for 32 minutes at 37ºC.

Technical Note:

Biocare's VP Echelon Series of predilutes have been developed for use with Ventana® Medical Systems, BenchMark® XT Immunohistochemistry Staining System in combination with Ventana® Detection Kits and Ventana® Prep Kit Dispensers.

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 listed are not applicable to other detection systems, as results may vary. Ultimately, it is the responsibility of the investigator to determine optimal conditions. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

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 in 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.

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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.
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.

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