Melanoma Cocktail
Concentrated and Prediluted Monoclonal Antibody
901-078-090817

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, prenal and infantile retinal pigment epithelium and melanoma cells. It is also thought to be oncotelic 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 melanomas. 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. A secondary antibody may be applied to bind the primary antibody, followed by an enzyme labeled polymer; or an enzyme labeled polymer may be applied directly to bind the primary antibody. The detection of the bound primary antibody is evidenced by an enzyme-mediated 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 + 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. 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.

Storage and Stability Cont’d:
Diluted reagents should be used promptly; any remaining reagent should be stored at 2ºC to 8ºC.

Protocol Recommendations (manual use):
Peroxide Block: Block for 5 minutes with Biocare’s Peroxidazed 1.
Pretreatment: Perform heat retrieval using Biocare’s Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.

Protein Block (Optional): Incubate for 5-10 minutes at RT with Biocare’s 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 Biocare’s Wap’s Red.

Counterstain: Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha’s Bluing Solution for 1 minute. Rinse with deionized water.

Protocol Recommendations (Ventana Benchmark ULTRA):
Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows: Template: UltraView DAB
Pretreatment Protocol: ULTRA CC1 Standard (64 min) at 95°C
Primary Antibody: Incubate for 32 minutes at 37°C

Detection: UltraView

Technical Note:
1. This antibody has been standardized with Biocare’s MACH 4 detection system. Use TBS buffer for washing steps.

Limitations:
The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to 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. 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:

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 (NaN3) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to

Catalog Number: CM 078 B, C
Description: 0.5, 1.0 ml, concentrated
Dilution: 1:100
Diluent: Van Gogh Yellow

Catalog Number: PM 078 AA
Description: 6.0 ml, prediluted
Dilution: Ready-to-use

Catalog Number: VP 078 G
Description: 6.0 ml, prediluted
Dilution: Ready-to-use

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Precautions Cont’d:
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
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
the detection of malignant melanoma in paraffin-embedded tissues.
morphologic and immunocytochemical analysis including application of
and carcinomas with ascites fluid preparations of antimelanoma
antibody (HMB-45) and other immunohistochemical 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.
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

*VP Echelon Series antibodies are developed solely by Biocare Medical
LLC and do not imply approval or endorsement of Biocare’s antibodies
by Ventana Medical Systems, Inc. Biocare and Ventana are not
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