**Melanoma Cocktail**

Concentrated and Prediluted Monoclonal Antibody 901-078-030618

<table>
<thead>
<tr>
<th>Catalog Number:</th>
<th>CM 078 B, C</th>
<th>PM 078 AA</th>
<th>VP 078 G</th>
</tr>
</thead>
<tbody>
<tr>
<td>Description:</td>
<td>0.5, 1.0 ml, concentrated</td>
<td>6.0 ml, prediluted</td>
<td>6.0 ml, prediluted</td>
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<tr>
<td>Dilution:</td>
<td>1:100</td>
<td>Ready-to-use</td>
<td>Ready-to-use</td>
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<tr>
<td>Diluent:</td>
<td>Van Gogh Yellow</td>
<td>N/A</td>
<td>N/A</td>
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</tbody>
</table>

**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. 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 2°C to 8°C. 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. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

**Protocol Recommendations (intelliPATH and manual use):**

- **Peroxide Block:** Block for 5 minutes with Biocare's Peroxidized 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 Warp 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 XT / ULTRA Slide Staining Systems):**

- Using ultraView 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

**Technical Note:**

This antibody, for intelliPATH and manual use, 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 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 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 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:

*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 affiliated, associated or related in any way. Ventana®, BenchMark®, ultraView and OptiView are trademarks of Roche.