Pan Melanoma Cocktail-2 (MART-1 and Tyrosinase)
Concentrated and Prediluted Monoclonal Antibody
901-178-031519

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CM 178 A</td>
<td>0.1 mL, conc.</td>
<td>Van Gogh Yellow</td>
<td>VLM178 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:</td>
<td>Peroxide Block: Block for 5 minutes with Peroxidase.</td>
<td>OAI178 is intended for use with the ONCORE. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:</td>
</tr>
<tr>
<td>PM 178 AA</td>
<td>6.0 mL, RTU</td>
<td>N/A</td>
<td>Deparaffinization: Deparaffinize for 8 minutes with Val DePar.</td>
<td>Pretreatment: Perform heat retrieval using Reveal Decloaker. Refer to the Reveal Decloaker product data sheet for specific instructions.</td>
<td>Protocol Name: Pan Mel 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Peroxidase Block: Block for 5 minutes with Val Peroxidase Block.</td>
<td>Protein Block (Optional): Incubate for 5-10 minutes at RT with Val Universal Linker.</td>
<td>Protocol Template (Description): Ms HRP Template 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Protein Block (Optional): Incubate for 10-20 minutes at RT with Val Background Block.</td>
<td>Primary Antibody: Incubate for 10 minutes with Val Mouse Secondary.</td>
<td>Deparaffinization: Deparaffinize for 8 minutes with Val DePar.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Polymer: Incubate for 10 minutes with Val Universal Polymer.</td>
<td>Chromogen: Incubate for 5 minutes with Val DAB.</td>
<td>Peroxidase Block: Block for 5 minutes with Peroxidase.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chromogen: Incubate for 5 minutes with Val DAB.</td>
<td>Counterstain: Counterstain for 5 minutes with Val Hematoxylin.</td>
<td>Protein Block (Optional): Incubate for 5-10 minutes at RT with Val Background Punisher.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Counterstain: Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Technical Note: This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ready-to-use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ready-to-use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ready-to-use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Intended Use:**
For In Vitro Diagnostic Use
Pan Melanoma Cocktail-2 (MART-1 and Tyrosinase) is a mouse monoclonal antibody cocktail that is intended for laboratory use in the qualitative identification of MART-1 and Tyrosinase 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:**
MART-1 recognizes a protein of 18 kDa, identified at MART-1 (Melanoma Antigen Recognized by T cells 1). MART-1 is a useful addition to melanoma panels as it is apparently specific for melanocytic lesions (1,4). Studies have shown that MART-1 is more sensitive than HMB45 when labeling metastatic melanomas (3). This MART-1 cocktail does not stain steroid tumors like Melan A [103] does. Tyrosinase is a key enzyme involved in the initial stages of melanin biosynthesis. Studies have shown Tyrosinase to be a more sensitive marker when compared to HMB45 and MART-1. It has also been shown to label a higher percentage of desmoplastic melanomas than HMB45 (1). The combination of MART-1 and Tyrosinase aids in identifying metastatic melanoma in sentinel lymph nodes (2).

**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:** M2-7C10 + M2-9E3 + T311

**Isotype:** IgG2b + IgG2b + IgG2a

**Protein Concentration:** Call for lot specific Ig concentration

**Epitope/Antigen:** MART-1 and Tyrosinase

**Cellular Localization:** Cytoplasmic

**Positive Tissue Control:** Melanoma

**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. 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 2°C to 8°C.
Pan Melanoma Cocktail-2 (MART-1 and Tyrosinase)
Concentrated and Prediluted Monoclonal Antibody
901-178-031519

Limitations Cont’d:
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:

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) (5)
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. (6)
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 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: