**Pan Melanoma Cocktail-2 (MART-1 and Tyrosinase)**

**Concentrated and Prediluted Monoclonal Antibody**

901-178-090517

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<table>
<thead>
<tr>
<th>Catalog Number</th>
<th>Description</th>
<th>Dilution</th>
<th>Diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM 178 A</td>
<td>0.1 ml, concentrated</td>
<td>1:100</td>
<td>Van Gogh Yellow</td>
</tr>
<tr>
<td>PM 178 AA</td>
<td>6.0 ml, prediluted</td>
<td>Ready-to-use</td>
<td>N/A</td>
</tr>
<tr>
<td>OAI 178 T60</td>
<td>60 tests, prediluted</td>
<td>Ready-to-use</td>
<td>N/A</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 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. 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:** M2-7C10 + M2-9E3 + T311

**Isotype:** IgG2b and IgG2b and IgG2a

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

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

**Cellular Localization:** Cytoplasmic

**Positive 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. 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 (manual use):**

**Peroxide Block:** Block for 5 minutes with Biocare’s Peroxidazed 1.

**Protocol Recommendations (manual use) Cont’d:**

**Pretreatment:** Perform heat retrieval using Biocare’s Reveal Decloaker. Refer to the Reveal Decloaker product 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 (ONCORE Automated Slide Staining System):**

OAI178 is intended for use with the ONCORE Automated Slide Staining System. Refer to the ONCORE Automated Slide Staining System User Manual for specific instructions on its use. Protocol parameters in the ONCORE Automated Slide Stainer Protocol Editor should be programmed as follows:

**Protocol Name:** Pan Mel 2

**Protocol Template (Description):** Ms HRP Template 1

**Dewaxing (DS Option):** DS2

**Antigen Retrieval (AR Option):** AR2, low pH; 101°C

**Reagent Name, Time, Temp.:** Pan Mel 2, 30 min., 25°C

**Technical Note:**

This antibody has been optimized for use with Biocare’s MACH 4 Universal HRP-Polymer Detection and ONCORE HRP Detection. 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 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 Precautions Cont’d:
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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:


