MART-1 + Tyrosinase + pH3
Prediluted Multiplex Antibody Reagent
901-3186DS-090817

Catalog Number: API 3186DS AA
Description: 6.0 ml, prediluted
Dilution: Ready-to-use
Diluent: N/A

Intended Use:
For In Vitro Diagnostic Use
MART-1 + Tyrosinase + pH3 is a cocktail of mouse and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of MART-1, Tyrosinase and phosphohistone H3 (Ser10) 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, 2). 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 has been shown to be a more sensitive marker in recognizing melanoma 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 (4). Microscopic evaluation of mitotic figures on H&E is a routine procedure in the assessment of the tumor grades (5). However, the counting of mitosis is manual and time consuming with assorted difficulties as well as variabilities between interobserver assessments (6). Histone H3 phosphorylation at Serine10 (pH3) is in association with mitotic chromatin condensation in late G2 and M phase of the cell cycle. pH3 can distinguish mitosis from apoptotic nuclei (7). The immunohistochemical staining of Serine10-pH3 has been reported to be comparable to mitotic figures in the H&E section (8-11). The combination of monoclonal anti-pH3 with MART-1 and Tyrosinase would offer an advantage of specific epitopes for melanoma diagnosis and mitosis counting.

Principle of Procedure:
This product is a primary antibody cocktail of mouse and rabbit antibodies, which may be used in a Multiplex IHC staining procedure to produce a two-color stain. Following application of the primary antibody cocktail to the tissue sample, detection is performed by separate secondary antibodies specific for each species (i.e. mouse or rabbit) of the primary antibody cocktail, which are conjugated to horseradish peroxidase (HRP) or alkaline phosphatase (AP) enzymes. Visualization is accomplished by the application of chromogenic substrates (DAB and Warp Red), which are enzymatically activated (by HRP or AP, respectively) to produce a colored reaction product at the antigen site. The specimen may be counterstained and coverslipped. Results are interpreted using a light microscope.

Reagent Provided:
MART-1 + Tyrosinase + pH3 is provided as a prediluted antibody cocktail of anti-MART-1, anti-Tyrosinase and anti-pH3 antibodies in buffer with carrier protein and preservative.

<table>
<thead>
<tr>
<th>Antibody</th>
<th>anti-MART-1</th>
<th>anti-Tyrosinase</th>
<th>anti-pH3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clone</td>
<td>M2-7C10 + M2-9E3</td>
<td>T311</td>
<td>BC37</td>
</tr>
<tr>
<td>Source</td>
<td>Mouse monoclonal</td>
<td>Mouse monoclonal</td>
<td>Rabbit monoclonal</td>
</tr>
<tr>
<td>Isotype</td>
<td>IgG2b</td>
<td>IgG2a</td>
<td>IgG6</td>
</tr>
<tr>
<td>Epitope/Antigen</td>
<td>MART-1</td>
<td>Tyrosinase</td>
<td>PhosphoSer10 of Histone H3</td>
</tr>
<tr>
<td>Cellular Localization</td>
<td>Cytoplasmic</td>
<td>Cytoplasmic</td>
<td>Nuclear (mitotic figure)</td>
</tr>
<tr>
<td>Staining</td>
<td>Red (Warp Red)</td>
<td>Red (Warp Red)</td>
<td>Brown (DAB)</td>
</tr>
</tbody>
</table>

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.

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Species Reactivity:
Human; others not tested

Positive Tissue Control:
Melanoma

Protocol Recommendations:

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:
Incubate for 10 minutes at RT with Biocare’s Background Punisher.

Primary Antibody:
Incubate for 30 minutes at RT.

Double Stain Detection:
Incubate for 30 minutes at RT using Biocare’s MACH 2 Double Stain 1.

Chromogen (1):
Incubate for 5-7 minutes at RT with Biocare’s Warp Red. Rinse in deionized water.

Chromogen (2):
Incubate for 5 minutes at RT with Biocare’s DAB.

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

Technical Note:
This antibody has been standardized with Biocare’s MACH 2 Double Stain 1. 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 presentations, 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 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) (12)
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. (13)
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: