# HMB45 + MART-1 + Tyrosinase

Prediluted Monoclonal Antibody
901-165-010923

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| Available Product Formats | | | | |
|---|---|---|---|---|
| Format | Catalog Number | Description | Dilution | Diluent |
| Q Series– For Leica BOND-III | ALI 165 G7 | 7.0 mL | Ready-to-use | N/A |

**Intended Use:**
For In Vitro Diagnostic Use
HMB45 + MART-1 + Tyrosinase is a mouse monoclonal antibody cocktail that is intended for laboratory use in the qualitative identification of HMB45 + MART-1 + 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:**
The HMB45 clone reacts with a neuraminidase-sensitive oligosaccharide side chain of a glycoconjugate present in immature melanosomes. Studies have shown 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.[1] The MART-1/Melan A recognizes a protein of 18kDa, identified as MART-1 (Melanoma Antigen Recognized by T cells 1) or Melan-A.[1] Melan-A is a useful addition to melanoma panels which is specific to melanocytic lesions. 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-1The combination of HMB45, MART-1 cocktail and Tyrosinase make this quadruple antibody cocktail a useful screening tool for melanoma.[1,2]

**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-step or two-step detection procedure can be applied. A one-step procedure will feature an enzyme labeled polymer that binds the primary antibody. A two-step procedure will feature a linker antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

**Reagent Provided:**
HMB45 + MART-1 + Tyrosinase is provided as a prediluted antibody cocktail of anti-HMB45, anti-MART-1, and anti-Tyrosinase antibodies, in buffer with carrier protein and preservative.

| **Antibody** | anti-HMB45 | anti-MART-1 | anti-Tyrosinase |
|---|---|---|---|
| **Clone** | HMB45 | M2-7C10 + M2-9E3 | T311 |
| **Source** | Mouse monoclonal | Mouse monoclonal | Mouse monoclonal |
| **Isotype** | IgG1/kappa | IgG2b + IgG2b | IgG2a |
| **Epitope/ Antigen** | HMB45 | MART-1 | Tyrosinase |
| **Cellular Localization** | Cytoplasmic | Cytoplasmic | Cytoplasmic |
| **Staining** | Brown (DAB) | Brown (DAB) | Brown (DAB) |

**Storage and Stability:**
Store at 2ºC to 8ºC. 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 2ºC to 8ºC.

**Known Applications:**
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
**Species Reactivity:** Human, others not tested
**Positive Tissue Control:** Metastatic melanoma

**Protocol Recommendations (Q Series – For Leica BOND-III):**
ALI165 is intended for use with the Leica BOND-III. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:
**Protocol Name:** IHC Protocol F + Blocker
**Detection:** Bond Polymer Refine
**HIER:** 10 min with ER1
**Peroxide Block:** 5 min
**Background Block:** 10 min
**Marker (Primary Antibody):** 15 min
**Post Primary:** 8 min
**Polymer:** 8 min
**Mixed DAB Refine:** 10 min
**Hematoxylin:** 5 min

**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.

**Quality Control:**
Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

**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 ($NaN_3$) 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)[3]
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.[4]
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:**
1. Orchard G. Evaluation of melanocytic neoplasms: application of a pan-melanoma antibody cocktail. Br J Biomed Sci. 2002;59(4):196-202.
2. Cook MG, *et al.* The development of optimal pathological assessment of sentinel lymph nodes for melanoma. J Pathol. 2003 Jul;200(3):314-9.
3. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."

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**References Cont'd:**
4. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.



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