

## HMB45 + MART-1 + Tyrosinase

Prediluted Cocktail Antibodies

Control Number: 901-165VP-090517

### VP Echelon™ Series

**Catalog Number:** VP 165 G

**Description:** 6.0, ml, prediluted

**Dilution:** Ready-to-use

#### Intended Use:

For In Vitro Diagnostic Use

HMB45 + MART-1 + Tyrosinase is intended for laboratory use in the qualitative identification of HMB45, 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:

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 (6). This antibody has been shown to label the majority of melanomas. 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 and is specific to melanocytic lesions. Studies have also shown that MART-1 is more sensitive than HMB45 when labeling metastatic melanomas. 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. The combination of HMB45, MART-1 and Tyrosinase make this quadruple antibody cocktail a first-order pan melanoma screener, and may prove to be a valuable marker for melanoma metastasis in sentinel lymph nodes (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, an enzyme labeled polymer is added to bind to the primary antibody. The detection of the bound antibody is 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 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.

#### Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Species Reactivity:** Human, others not tested

**Positive Tissue Control:** Metastatic melanoma

#### Protocol Recommendations:

Using *ultraVIEW™* Detection Kit

**Pretreatment Solution (recommended):** CC1

**Pretreatment Protocol:** Standard

**Primary Antibody:** Incubate for 32 minutes at 37°C.

#### Technical Note:

Biocare's VP Echelon Series of predilutes have been developed for use with Ventana® Medical Systems, BenchMark® XT Immunohistochemistry Staining System in combination with Ventana® Detection Kits and Ventana® Prep Kit Dispensers.

#### 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:

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<sub>3</sub>) 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 in 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.

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### References:

1. Orchard G. Evaluation of melanocytic neoplasms: application of a pan-melanoma antibody cocktail. *Br J Biomed Sci.* 2002;59(4):196-20.
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."
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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