

# HMB45 + MART-1 + Tyrosinase

Concentrated and Prediluted Cocktail Antibodies  
901-165-052523

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M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	CM 165 B, C	0.5, 1.0 mL	1:100	Van Gogh Yellow
Predilute	PM 165 AA, H	6.0, 25 mL	Ready-to-use	N/A
intelliPATH FLX	IPI 165 G10	10 mL	Ready-to-use	N/A
ONCORE Pro	OPAI 165 T60	60 tests	Ready-to-use	N/A
UltraLine – For BenchMark	VP 165 G, G25	6.0, 25mL	Ready-to-use	N/A

## 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 (1). 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 which 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 cocktail 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, 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.

## Reagent Provided:

HMB45 + MART-1 + Tyrosinase is provided as a concentrated or 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. 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.

## Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

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

**Positive Tissue Control:** Metastatic melanoma

## Protocol Recommendations (VALENT® Automated Slide Staining Platform):

VLTM165 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:

### - DAB Chromogen Staining Option:

**Deparaffinization:** Deparaffinize for 8 minutes with Val DePar.

**Pretreatment:** Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X- OR – Val AR-Lo pH, 5X (use at 1X).

**Peroxidase Block:** Block for 5 minutes with Val Peroxidase Block.

**Protein Block (Optional):** Incubate for 10-20 minutes at RT with Val Background Block.

**Primary Antibody:** Incubate for 30 minutes.

**Secondary:** Incubate for 10 minutes with Val Mouse Secondary.

**Linker:** Incubate for 10 minutes with Val Universal Linker.

**Polymer:** Incubate for 10 minutes with Val Universal Polymer.

**Chromogen:** Incubate for 5 minutes with Val DAB.

**Counterstain:** Counterstain for 5 minutes with Val Hematoxylin.

### - Red Chromogen Staining Option:

**Deparaffinization:** Deparaffinize for 8 minutes with Val DePar.

**Pretreatment:** Perform heat retrieval at 98°C for 60 minutes using Val AR-Hi pH, 5X (use at 1X).

**Protein Block (Optional):** Incubate for 10-20 minutes with Val Background Block.

**Primary Antibody:** Incubate for 30 minutes.

**Polymer:** Incubate for 45 min with Val Mouse AP Polymer.

**Chromogen:** Incubate for 15 min with Val Fast Red.

**Counterstain:** Counterstain for 5 minutes with Val Hematoxylin.

## Protocol Recommendations (intelliPATH FLX® and manual use):

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**Peroxide Block:** Block for 5 minutes with Peroxidized 1.

**Pretreatment:** Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.

**Protein Block:** Incubate for 10 minutes at RT with 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 Betazoid DAB -OR- Incubate for 5-7 minutes at RT with Warp Red. Rinse in deionized water.

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

## **intelliPATH™ Automated Slide Stainer:**

IPI165 is intended for use on the intelliPATH FLX. Refer to the User Manual for specific instructions for use. When using the intelliPATH FLX, peroxide block with intelliPATH FLX Peroxidase Blocking Reagent (IPB5000) may be performed following heat retrieval.

### **Technical Note:**

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

## **Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System):**

OPA1165 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

**Protocol Name:** HM/MT1/Ty – OR – HM/MT1/Ty AP

**Protocol Template (Description):** Ms HRP Template 1 – OR – Ms AP Template 1

**Dewaxing (DS Buffer Option):** DS2-50

**Antigen Retrieval (AR Option):** AR2, low pH; 103°C

**Block Option:** Buffer

**Reagent Name, Time, Temp.:** HM/MT1/Ty, 30 min., 25°C

## **Protocol Recommendations (Ventana BenchMark XT / ULTRA):**

VP165 is intended for use with the BenchMark XT / ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

- Using **ultraView on XT / ULTRA:**

**Template/Detection:** ultraView DAB

**Pretreatment Protocol:** CC1 Standard

**Primary Antibody:** 32 minutes, 37°C

- Using **OptiView on ULTRA:**

**Template/Detection:** OptiView DAB IHC

**Pretreatment Protocol:** CC1 32 minutes

**Peroxidase:** Pre Primary Peroxidase Inhibitor

**Primary Antibody:** 16 minutes, 36°C

## **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](http://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.

### **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."

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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