HMB45 + MART-1 + Tyrosinase

Concentrated and Prediluted Monoclonal Antibody
902-165-092821

BIOCARE MEDICAL

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	APR 165 B, C	0.5, 1.0 mL	1:100	Van Gogh Yellow
Predilute	APR 165 AA, H	6.0, 25 mL	Ready-to-use	N/A
UltraLine – For BenchMark	AVR 165 G, G25	6.0, 25 mL	Ready-to-use	N/A

Intended Use:
For Research Use Only. Not for use in diagnostic procedures.

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 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.

Staining Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 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:
IPR165 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.

Staining Protocol Recommendations (Ventana BenchMark ULTRA):
AVR165 is intended for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

- Using ultraView on 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:
This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

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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.

Technical Support:

Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

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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