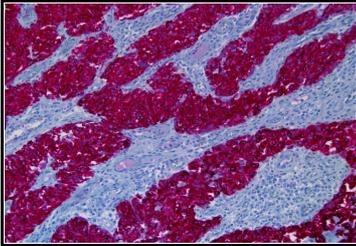


## Melanoma Triple Cocktail (HMB45+A103+T311) Primary Antibody

**REF** 790-4677  
06527787001

**IVD**  50



**Figure 1. Melanoma Triple Cocktail (HMB45+A103+T311) cytoplasmic staining of melanoma.**

### INTENDED USE

Melanoma Triple Cocktail (HMB45+A103+T311) is an antibody cocktail of anti-Melanosome (HMB45), anti-MART-1/melan A (A103), and anti-Tyrosinase (T311) mouse monoclonal antibodies. This antibody cocktail demonstrates cytoplasmic staining and may be used to aid in the identification of melanoma. The antibody is intended for qualitative staining in sections of formalin-fixed, paraffin-embedded tissue.

This product should be interpreted by a qualified pathologist in conjunction with histological examination, relevant clinical information, and proper controls.

This antibody is intended for *in vitro* diagnostic (IVD) use.

### SUMMARY AND EXPLANATION

Melanoma Triple Cocktail (HMB45+A103+T311) is an antibody cocktail of anti-Melanosome (HMB45), anti-MART-1/melan A (A103), and anti-Tyrosinase (T311) mouse monoclonal antibodies. MART-1/melan A, melanosome, and tyrosinase are melanocyte markers and are expressed in the majority of malignant melanocytic lesions.<sup>1,2,3</sup> Each of the individual antibodies in the cocktail demonstrates cytoplasmic staining and thus will overlay on tissues where two or more antibodies stain, allowing a potential for increased sensitivity. A cocktail of antibodies directed against MART-1/melan A, melanosome, and tyrosinase has been reported to provide advantages in sensitivity, over the use of the individual antibodies alone, in the detection of melanoma, while still maintaining good specificity.<sup>4</sup> In addition, in cases of lymph node and cutaneous metastases this cocktail was as sensitive as anti-S100.<sup>4</sup>

### REAGENT PROVIDED

Melanoma Triple Cocktail (HMB45+A103+T311) contains sufficient reagent for 50 tests.

One 5 mL dispenser of Melanoma Triple Cocktail (HMB45+A103+T311) contains approximately 23.5 µg of a mouse monoclonal antibody cocktail.

The antibody is diluted in 0.05M Tris-HCL dilution buffer with 2% carrier protein and 0.10% ProClin 300, a preservative

Total protein concentration of the reagent is approximately 10mg/mL. Specific antibody concentration is approximately 4.7 µg/mL. There is no known non-specific antibody reactivity observed in this product.

Melanoma Triple Cocktail (HMB45+A103+T311) is a cocktail of monoclonal antibodies produced as a cell culture supernatant.

Refer to the appropriate VENTANA detection kit package insert for detailed descriptions of: (1) Principles of the Procedure, (2) Materials and Reagents Needed but Not Provided, (3) Specimen Collection and Preparation for Analysis, (4) Quality Control Procedures, (5) Troubleshooting, (6) Interpretation of Results, and (7) General Limitations.

### MATERIALS REQUIRED BUT NOT PROVIDED

Staining reagents, such as VENTANA detection kits and ancillary components, including negative and positive tissue control slides, are not provided.

### STORAGE

Store at 2-8°C. Do not freeze.

To ensure proper reagent delivery and the stability of the antibody, replace the dispenser cap after every use and immediately place the dispenser in the refrigerator in an upright position.

Every antibody dispenser is expiration dated. When properly stored, the reagent is stable to the date indicated on the label. Do not use reagent beyond the expiration date.

### SPECIMEN PREPARATION

Routinely processed, formalin-fixed, paraffin-embedded tissues are suitable for use with this primary antibody when used with VENTANA detection kits and a VENTANA BenchMark XT and BenchMark ULTRA automated slide stainer. The recommended tissue fixative is 10% neutral buffered formalin.<sup>5</sup> Slides should be stained immediately, as antigenicity of cut tissue sections may diminish over time.

It is recommended that positive and negative controls be run simultaneously with unknown specimens.

### WARNINGS AND PRECAUTIONS

- For *in vitro* diagnostic (IVD) use.
- ProClin 300 is used as a preservative in this solution. It is classified as an irritant and may cause sensitization through skin contact. Take reasonable precautions when handling. Avoid contact of reagents with eyes, skin, and mucous membranes. Use protective clothing and gloves.
- Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
- Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
- Avoid microbial contamination of reagents as it may cause incorrect results.
- Consult local and/or state authorities with regard to recommended method of disposal.
- For supplementary safety information, refer to the product Safety Data Sheet and the Symbol and Risk Phrase Guide located at [www.ventana.com](http://www.ventana.com).

### STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on VENTANA BenchMark XT and BenchMark ULTRA automated slide stainers in combination with VENTANA *ultraView* Universal Alkaline Phosphatase Red Detection Kit and accessories. Refer to Table 1 for recommended staining protocols.

This antibody has been optimized for specific incubation times but the user must validate results obtained with this reagent.

The parameters for the automated procedures can be displayed, printed and edited according to the procedure in the instruments Operator's Manual. Refer to the appropriate VENTANA detection kit package insert for more details regarding immunohistochemistry staining procedures.

**Table 1.** Recommended Staining Protocol for Melanoma Triple Cocktail (HMB45+A103+T311) with VENTANA *ultraView* Universal Alkaline Phosphatase Red Detection Kit on a BenchMark XT instrument and BenchMark ULTRA instrument.

Procedure Type	Method
Deparaffinization	Selected
Cell Conditioning (Antigen Unmasking)	Cell Conditioning 1, Standard
Antibody (Primary)	BenchMark XT instrument 16 minutes, 37°C BenchMark ULTRA instrument 32 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes
Post Counterstain	Bluing, 4 minutes

Due to variation in tissue fixation and processing, as well as general lab instrument and environmental conditions, it may be necessary to increase or decrease the primary antibody incubation, cell conditioning or protease pretreatment based on individual



specimens, detection used, and reader preference. For further information on fixation variables, refer to "Immunohistochemistry Principles and Advances".<sup>6</sup>

**POSITIVE TISSUE CONTROL**

Examples of positive control tissues for Melanoma Triple Cocktail (HMB45+A103+ T311) are primary melanoma tumor cells and melanocytes in normal skin.

**STAINING INTERPRETATION**

The cellular staining pattern for Melanoma Triple Cocktail (HMB45+A103+T311) is cytoplasmic.

**SPECIFIC LIMITATIONS**

Necrosis often present in melanomas may stain non-specifically. If non-specific staining is detected in the negative control, the addition of a blocking agent may be used.

**PERFORMANCE CHARACTERISTICS**

Staining tests for specificity, sensitivity, and repeatability were conducted and the results listed in Table 2 and Table 3 and in the Repeatability section.

**Specificity**

**Table 2.** Specificity of the Melanoma Triple Cocktail (HMB45+A103+T311) was determined by testing formalin-fixed, paraffin-embedded normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Thymus	0/3
Cerebellum	0/3	Myeloid (bone marrow)	0/3
Adrenal gland	1/3	Lung	0/3
Ovary	0/3	Heart	0/3
Pancreas	0/3	Esophagus	0/3
Parathyroid gland	0/3	Stomach	0/3
Pituitary gland	3/3	Small intestine	0/3
Testis	0/3	Colon	0/3
Thyroid	0/3	Liver	0/3
Breast	0/3	Salivary gland	0/3
Spleen	0/3	Kidney	0/3
Tonsil	0/3	Prostate	0/3
Uterus	0/3	Skin	2/3
Striated muscle	0/3	Mesothelium and lung	0/3
Nerve (sparse)	0/3		

**Sensitivity**

**Table 3.** Sensitivity of Melanoma Triple Cocktail (HMB45+A103+T311) was determined by testing a variety of formalin-fixed, paraffin-embedded neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma	0/1
Atypical meningioma	0/1
Malignant ependymoma	0/1
Malignant oligodendroglioma	0/1
Serous papillary adenocarcinoma	0/1

Pathology	# positive / total cases
Mucinous papillary adenocarcinoma	0/1
Islet cell carcinoma	0/1
Pancreatic adenocarcinoma	0/1
Seminoma	0/1
Embryonal carcinoma	0/1
Medullary carcinoma	0/1
Papillary carcinoma	0/1
Breast intraductal carcinoma	0/2
Breast invasive ductal carcinoma	0/1
Diffuse large B-cell lymphoma	0/2
Lung small cell undifferentiated carcinoma	0/1
Lung squamous cell carcinoma	0/1
Lung adenocarcinoma	0/1
Esophageal squamous cell carcinoma	0/1
Esophageal adenocarcinoma	0/1
Gastric mucinous adenocarcinoma	0/1
Gastrointestinal adenocarcinoma	0/2
Intestinal interstitialoma	0/1
Abdominal malignant interstitialoma	0/1
Rectal Adenocarcinoma	0/1
Rectal interstitialoma	0/1
Hepatocellular carcinoma	0/1
Hepatoblastoma	0/1
Renal clear cell carcinoma	0/1
Prostatic adenocarcinoma	0/2
Leiomyoma	0/1
Endometrial adenocarcinoma	0/1
Endometrial clear cell carcinoma	0/1
Uterine squamous cell carcinoma	0/1
Embryonal rhabdomyosarcoma	0/1
Basal cell carcinoma	0/1
Squamous cell carcinoma	0/1
Neurofibroma	0/1
Ganglioneuroblastoma	0/1
Epithelial malignant mesothelioma	0/1
Diffuse malignant lymphoma	0/1
Hodgkin's lymphoma	0/1
Diffuse malignant lymphoma	0/1

Pathology	# positive / total cases
Bladder transitional cell carcinoma	0/1
Low grade leiomyosarcoma	0/1
Osteosarcoma	0/1
Spindle cell rhabdomyosarcoma	0/1
Intermediate grade leiomyosarcoma	0/1
Malignant melanoma	214/311

**CONTACT INFORMATION**


Ventana Medical Systems, Inc.  
1910 E. Innovation Park Drive  
Tucson, Arizona 85755  
USA  
+1 520 887 2155  
+1 800 227 2155 (USA)



[www.ventana.com](http://www.ventana.com)



Roche Diagnostics GmbH  
Sandhofer Strasse 116  
D-68305 Mannheim  
Germany

**Repeatability**

Repeatability studies for Melanoma Triple Cocktail (HMB45+A103+T311) were completed to demonstrate:

- Inter-lot reproducibility of the antibody.
- Intra-run and Inter-run reproducibility on a BenchMark XT instrument.
- Intra-platform reproducibility on the BenchMark XT instrument and the BenchMark ULTRA instrument.
- Inter-platform reproducibility between the BenchMark XT instrument and BenchMark ULTRA instrument.

All studies met their acceptance criteria.

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