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

REF 790-4677

06527787001

IVD Σ 50

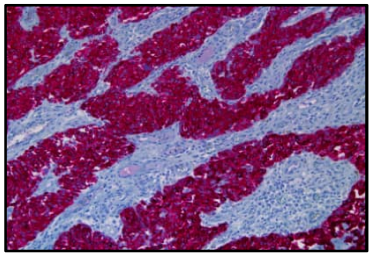

Figure 1. Melanoma Triple Cocktail (HMB45+A103+T311) antibody cytoplasmic staining of melanoma.

INTENDED USE

Melanoma Triple Cocktail (HMB45+A103+T311) is a cocktail of anti-Melanosome (HMB45), anti-MART-1/melan A (A103), and anti-Tyrosinase (T311) mouse monoclonal antibodies. Melanoma Triple Cocktail (HMB45+A103+T311) is intended for laboratory use in the qualitative immunohistochemical detection of premelanosome protein (PMEL), melan A, and tyrosinase by light microscopy in sections of formalin-fixed, paraffin-embedded tissue stained on a BenchMark IHC/ISH instrument.

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) Primary Antibody (Melanoma Triple Cocktail (HMB45+A103+T311) antibody) is an antibody cocktail of anti-Melanosome (HMB45), anti-MART-1/melan A (A103), and anti-Tyrosinase (T311) mouse monoclonal antibodies. Premelanosome protein (PMEL), melan A, and tyrosinase are melanocyte markers and are expressed in the majority of melanocytic lesions.[1,2,3] A cocktail of antibodies directed against premelanosome protein (PMEL), melan A, and tyrosinase has been reported to provide advantages in sensitivity, over the use of the individual antibodies alone, in the detection of melanocytic lesions, while still maintaining good specificity.[4] In addition, in cases of lymph node and cutaneous metastases this cocktail was as sensitive as anti-S100.[4]

Melanoma Triple Cocktail (HMB45+A103+T311) antibody may be used as a melanocyte marker to aid in the differential diagnosis of melanocytic versus non-melanocytic tumors. The staining pattern is cytoplasmic.

PRINCIPLE OF THE PROCEDURE

Melanoma Triple Cocktail (HMB45+A103+T311) antibody is directed against MART-1/melan A, melanosome, and tyrosinase in formalin-fixed, paraffin-embedded (FFPE) tissue sections. This antibody can be visualized using *ultra*View Universal Alkaline Phosphatase Red Detection Kit (Cat. No. 760-501 / 05269814001). Refer to the *ultra*View Universal Alkaline Phosphatase Red Detection Kit method sheet for further information.

MATERIAL PROVIDED

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

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

The antibody cocktail is diluted in Tris-HCl dilution buffer with a carrier protein and 0.10% ProClin 300, a preservative.

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) antibody is a cocktail of monoclonal antibodies produced as cell culture supernatant.

Refer to the appropriate VENTANA detection kit method sheet for detailed descriptions of: Principle of the Procedure, Material and Methods, Specimen Collection and Preparation for Analysis, Quality Control Procedures, Troubleshooting, Interpretation of Results, and 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.

Not all products listed in the method sheet may be available in all geographies. Consult your local support representative.

The following reagents and materials may be required for staining but are not provided:

1. Recommended control tissue
2. Microscope slides, positively charged
3. Negative Control (Monoclonal) (Cat. No. 760-2014 / 05266670001).
4. *ultra*View Universal Alkaline Phosphatase Red Detection Kit (760-501 / 05269814001)
5. EZ Prep Concentrate (10X) (Cat. No. 950-102 / 05279771001)
6. Reaction Buffer Concentrate (10X) (Cat. No. 950-300 / 05353955001)
7. LCS (Predilute) (Cat. No. 650-010 / 05264839001)
8. ULTRA LCS (Predilute) (Cat. No. 650-210 / 05424534001)
9. Cell Conditioning Solution (CC1) (Cat. No. 950-124 / 05279801001)
10. ULTRA Cell Conditioning Solution (ULTRA CC1) (Cat. No. 950-224 / 05424569001)
11. Hematoxylin II (Cat. No. 790-2208 / 05277965001)
12. Bluing Reagent (Cat. No. 760-2037 / 05266769001)
13. Permanent mounting medium
14. Cover glass
15. Automated coverslipper
16. General purpose laboratory equipment
17. BenchMark IHC/ISH instrument

STORAGE AND STABILITY

Upon receipt and when not in use, 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 FFPE tissues are suitable for use with this primary antibody when used with VENTANA detection kits and BenchMark IHC/ISH instruments. The recommended tissue fixative is 10% neutral buffered formalin.[5] Sections should be cut at approximately 4 µm in thickness and mounted on positively charged slides. 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

1. For in vitro diagnostic (IVD) use.
2. For professional use only.
3. Do not use beyond the specified number of tests.
4. ProClin 300 solution is used as a preservative in this reagent. 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.
5. Positively charged slides may be susceptible to environmental stresses resulting in inappropriate staining. Ask your Roche representative for more information on how to use these types of slides.
6. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions. In the event of exposure, the health directives of the responsible authorities should be followed.[6,7]
7. Avoid contact of reagents with eyes and mucous membranes. If reagents come in contact with sensitive areas, wash with copious amounts of water.
8. Avoid microbial contamination of reagents as it may cause incorrect results.

9. For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and instructions for use of all necessary components located at navifyportal.roche.com.
10. Consult local and/or state authorities with regard to recommended method of disposal.
11. Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
12. To report suspected serious incidents related to this device, contact the local Roche representative and the competent authority of the Member State or Country in which the user is established.

This product contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
Warning 	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P261	Avoid breathing mist or vapours.
	P273	Avoid release to the environment.
	P280	Wear protective gloves.
	P333 + P313	If skin irritation or rash occurs: Get medical advice/ attention.
	P362 + P364	Take off contaminated clothing and wash it before reuse.
	P501	Dispose of contents/ container to an approved waste disposal plant.

This product contains CAS # 55965-84-9, reaction mass of: 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

STAINING PROCEDURE

VENTANA primary antibodies have been developed for use on BenchMark IHC/ISH instruments in combination with VENTANA detection kits and accessories. Refer to Table 2 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 instrument User Guide. Refer to the appropriate VENTANA detection kit method sheet for more details regarding immunohistochemistry staining procedures.

Table 2. Recommended staining protocol for Melanoma Triple Cocktail (HMB45+A103+T311) antibody with *ultra*View Universal Alkaline Phosphatase Red Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	XT	ULTRA or ULTRA PLUS[a]
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Standard	ULTRA CC1, 64 minutes, 95°C
Antibody (Primary)	16 minutes, 37°C	32 minutes, 36°C
Counterstain	Hematoxylin II, 4 minutes	
Post Counterstain	Bluing, 4 minutes	

[a] Concordance was demonstrated between BenchMark ULTRA and BenchMark ULTRA PLUS instruments using representative assays.

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."[8]

NEGATIVE REAGENT CONTROL

In addition to staining with Melanoma Triple Cocktail (HMB45+A103+T311) antibody, a second slide should be stained with the appropriate negative control reagent.

POSITIVE TISSUE CONTROL

Optimal laboratory practice is to include a positive control section on the same slide as the test tissue. This helps identify any failures applying reagents to the slide. Tissue with weak positive staining is best suited for quality control. Control tissue may contain both positive and negative staining elements and serve as both the positive and negative control. Control tissue should be fresh autopsy, biopsy, or surgical specimen, prepared or fixed as soon as possible in a manner identical to test sections.

Known positive tissue controls should be utilized only for monitoring performance of reagents and instruments, not as an aid in determining specific diagnosis of test samples. If the positive tissue controls fail to demonstrate positive staining, results of the test specimen should be considered invalid.

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

STAINING INTERPRETATION / EXPECTED RESULTS

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

All assays might not be registered on every instrument. Please contact your local Roche representative for more information.

PERFORMANCE CHARACTERISTICS

ANALYTICAL PERFORMANCE

Staining tests for specificity, sensitivity, and precision were conducted and the results are listed below.

Sensitivity and Specificity

Table 3. Sensitivity/Specificity of Melanoma Triple Cocktail (HMB45+A103+T311) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Heart	0/3
Cerebellum	0/3	Esophagus	0/3
Adrenal gland	1/3	Stomach	0/3
Ovary	0/3	Small intestine	0/3
Pancreas	0/3	Colon	0/3
Lymph node [a]	0/14	Liver	0/3
Parathyroid gland	0/3	Salivary gland	0/3
Pituitary gland	3/3	Kidney	0/3
Testis	0/3	Prostate	0/3
Thyroid	0/3	Endometrium	0/3
Breast	0/3	Cervix	0/3
Spleen	0/3	Skeletal muscle	0/2
Tonsil[a]	0/9	Skin	50/67
Thymus	0/3	Nerve	0/3
Bone marrow	0/3	Mesothelium	0/3

Tissue	# positive / total cases	Tissue	# positive / total cases
Lung	0/3		

[a] plasma cells staining

Table 4. Sensitivity/Specificity of Melanoma Triple Cocktail (HMB45+A103+T311) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	0/1
Meningioma (Cerebrum)	0/1
Ependymoma (Cerebrum)	0/1
Oligodendroglioma (Cerebrum)	0/1
Serous adenocarcinoma (Ovary)	0/1
Mucinous adenocarcinoma (Ovary)	0/1
Neuroendocrine neoplasm (Pancreas)	0/1
Adenocarcinoma (Pancreas)	0/1
Seminoma (Testis)	0/1
Embryonal carcinoma (Testis)	0/1
Medullary carcinoma (Thyroid)	0/1
Papillary carcinoma (Thyroid)	0/1
Ductal carcinoma in situ (Breast)	0/1
Microinvasive ductal carcinoma (Breast)	0/1
Invasive ductal carcinoma (Breast)	0/1
Small cell carcinoma (Lung)	0/1
Squamous cell carcinoma (Lung)	0/1
Adenocarcinoma (Lung)	0/1
Squamous cell carcinoma (Esophagus)	0/1
Adenocarcinoma (Esophagus)	0/1
Mucinous adenocarcinoma (Stomach)	0/1
Gastrointestinal stromal tumor (GIST) (Small intestine)	0/1
Adenocarcinoma (Colon)	0/2
Gastrointestinal stromal tumor (GIST) (Abdominal cavity)	0/1
Adenocarcinoma (Rectum)	0/1
Gastrointestinal stromal tumor (GIST) (Rectum)	0/1
Hepatocellular carcinoma (Liver)	0/1
Hepatoblastoma (Liver)	0/1
Renal cell carcinoma, unclassified (Kidney)	0/1
Adenocarcinoma (Prostate)	0/2
Leiomyoma (Uterus)	0/1
Adenocarcinoma (Uterus)	0/1
Clear cell carcinoma (Uterus)	0/1
Squamous cell carcinoma (Cervix)	0/2
Embryonal rhabdomyosarcoma (Striated muscle)	0/1
Basal cell carcinoma (Skin)	0/1
Squamous cell carcinoma (Skin)	0/1
Melanoma[a]	224/264
Nevus (Skin)	8/8
Intraepidermal nevus (Skin)	3/3
Intradermal nevus (Skin)	6/10
Compound nevus (Skin)	5/11
Junctional nevus (Skin)	1/2
Nevus Sebaceous (Skin)	0/1
Neurofibroma (Soft tissue)	0/1
Ganglioneuroblastoma (Retroperitoneum)	0/1
Spindle cell rhabdomyosarcoma (Retroperitoneum)	0/1
Mesothelioma (Abdominal cavity)	0/1
Hodgkin lymphoma (Lymph node)	0/1
Lymphoma, NOS	0/1
Diffuse large B-cell lymphoma (DLBCL)	0/2
B-cell Lymphoma, NOS	0/1
Urothelial carcinoma (Bladder)	0/1
Leiomyosarcoma (Bladder)	0/1
Osteosarcoma (Bone)	0/1
Leiomyosarcoma (Smooth muscle)	0/1

[a] Various organ sites were tested including but not limited to skin, lymph node and rectum

Precision

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

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark XT instrument.
- Between instrument precision on the BenchMark XT and BenchMark ULTRA instrument.
- Between platform precision between the BenchMark XT and BenchMark ULTRA instrument.

All studies met their acceptance criteria.

Precision on the BenchMark ULTRA PLUS instrument was demonstrated using representative assays. Studies include Within-run Repeatability, Between-day and Between-run Intermediate Precision. All studies met their acceptance criteria.

CLINICAL PERFORMANCE

Clinical performance data relevant to the intended purpose of Melanoma Triple Cocktail (HMB45+A103+T311) antibody were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

REFERENCES

1. Chen YT, Stockert E, Jungbluth A, et al. Serological analysis of Melan-A (MART-1), a melanocyte-specific protein homogeneously expressed in human melanomas. Proc Natl Acad Sci USA. 1996;93:5915-5919.
2. Duray PH, Palazzo J, Gown AM, et al. Melanoma cell heterogeneity. A study of two monoclonal antibodies compared with S-100 in paraffin sections. Cancer. 1988;61:2460-2468.
3. Hofbauer GF, Kamarashev J, Geertsen R et al. Tyrosinase immunoreactivity in formalin-fixed, paraffin-embedded primary and metastatic melanoma: frequency and distribution. J Cutan Pathol. 1998;25:204-209.

4. Orchard G. Evaluation of melanocytic neoplasms: application of a pan-melanoma antibody cocktail. Br J Biomed Sci. 2002;59:196-202.
5. Carson FL, Cappellano C. Histotechnology; A Self-Instructional Text, 5th edition. American Society for Clinical Pathology Press; 2020, 2022.
6. Occupational Safety and Health Standards: Occupational exposure to hazardous chemicals in laboratories. (29 CFR Part 1910.1450). Fed. Register.
7. Directive 2000/54/EC of the European Parliament and Council of 24 June 2020 on the protection of workers from risks related to exposure to biological agents at work.
8. Roche PC, Hsi ED. Immunohistochemistry-Principles and Advances. Manual of Clinical Laboratory Immunology, 6th edition. In: NR Rose, ed. ASM Press; 2002.

NOTE: A point (period/stop) is always used in this document as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

The summary of safety and performance can be found here:

https://ec.europa.eu/tools/eudamed

Symbols

Ventana uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see elabdoc.roche.com/symbols for more information).

GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

REVISON HISTORY

Rev	Updates
E	Updates to Warning and Precautions sections. Updated to current template.

INTELLECTUAL PROPERTY

For USA: Rx only

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