

CONFIRM anti-Melanosome (HMB45) Mouse Monoclonal Primary Antibody

REF 790-4366
05479282001

IVD 50

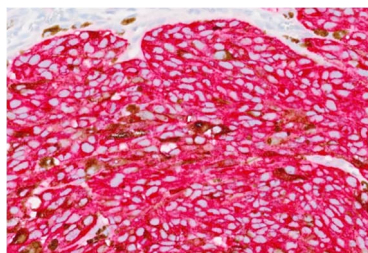


Figure 1. CONFIRM anti-Melanosome (HMB45) antibody staining on melanoma.

INTENDED USE

CONFIRM anti-Melanosome (HMB45) Mouse Monoclonal Primary Antibody is intended for laboratory use in the qualitative immunohistochemical detection of premelanosome protein (PMEL) 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

Detection of PMEL by immunohistochemistry (IHC) with the CONFIRM anti-Melanosome (HMB45) Mouse Monoclonal Primary Antibody (CONFIRM anti-Melanosome (HMB45) antibody) may be used as a melanocyte marker to aid in the differential diagnosis of melanocytic versus non-melanocytic tumors. It may be used as part of a panel of IHC studies. The staining pattern is cytoplasmic.

Human melanoma black (HMB45) is a monoclonal antibody raised against a 100 kDa type 1 transmembrane glycoprotein, premelanosome protein (PMEL). PMEL was discovered in numerous contexts, and as a result, has multiple aliases, the most common being: gp100, PMEL17, SILV, and SI.^{1,2} PMEL is responsible for the formation of fibrillar sheets within early stage melanosomes.^{3,4} The fibrillar sheets serve as a template upon which melanin polymers are deposited in later stage melanosomes.^{2,4} Alternative splicing leads to the generation of four PMEL isoforms, all of which react with monoclonal antibodies commonly used for IHC assays.²⁻⁷ Varying degrees of hypopigmentation and pigment cell inviability are seen in animals that either lack PMEL expression or express mutant PMEL variants, indicating that PMEL fibrils are required for optimal pigment cell function.^{3,4,8,9}

PMEL expression is restricted to cells of the melanocytic lineage. Cutaneous fetal melanocytes and prenatal and infantile retinal pigmented epithelium express PMEL.^{10,11} Whereas, cells of intradermal nevi or normal adult melanocytes do not, regardless of the degree of pigmentation.^{12,13} PMEL is expressed in the cytoplasm of neoplastic melanocytic cells, including the cells of junctional nevi and malignant melanoma.¹³ It is suggested that antibodies specific for PMEL recognize epitopes expressed only in proliferating melanocytes, both benign and malignant, which explains why fetal and malignant melanocytes, which are proliferative cells, react with PMEL antibodies while adult melanocytes are unreactive.¹³ Additional studies indicate that PMEL immunolabeling is reduced in mature melanosomes, suggesting that deposition of melanin in later stage melanosomes masks the PMEL epitopes on the fibrillar sheets.^{2,4}

PRINCIPLE OF THE PROCEDURE

CONFIRM anti-Melanosome (HMB45) antibody binds to melanosomes in active melanocytes. This antibody can be visualized using *ultraView* Universal Alkaline Phosphatase Red Detection Kit (Cat. No. 760-501 / 05269814001). Refer to the respective method sheet for further information.

MATERIAL PROVIDED

CONFIRM anti-Melanosome (HMB45) antibody contains sufficient reagent for 50 tests.

One 5 mL dispenser of CONFIRM anti-Melanosome (HMB45) antibody contains approximately 7.0 µg of a mouse monoclonal antibody.

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

Specific antibody concentration is approximately 1.4 µg/mL. There is no known non-specific antibody reactivity observed in this product.

CONFIRM anti-Melanosome (HMB45) Mouse Monoclonal Primary Antibody is a recombinant mouse monoclonal antibody produced as a purified 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. *ultraView* Universal Alkaline Phosphatase Red Detection Kit (Cat. No. 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. General purpose laboratory equipment
14. 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 formalin-fixed, paraffin-embedded (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.¹⁴ 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. Ask your Roche representative for a copy of "Recommended Slide Storage and Handling" for more information.

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

WARNINGS AND PRECAUTIONS

- For in vitro diagnostic (IVD) use.
- For professional use only.
- Do not use beyond the specified number of tests.
- 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.
- 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.
- 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.^{15,16}
- 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.
- For further information on the use of this device, refer to the BenchMark IHC/ISH instrument User Guide, and the instructions for use of all necessary components located at navifyportal.roche.com.
- Consult local and/or state authorities with regard to recommended method of disposal.
- Product safety labeling primarily follows EU GHS guidance. Safety data sheet available for professional user on request.
- 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 antibody or assay contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Table 1. Hazard information.

Hazard	Code	Statement
	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.

For more details on the proper use of this device, refer to the inline dispenser method sheet associated with P/N 790-4366.

Table 2. Recommended staining protocol for CONFIRM anti-Melanosome (HMB45) antibody with *ultraView* Universal Alkaline Phosphatase Red Detection Kit on BenchMark IHC/ISH instruments.

Procedure Type	Method	
	GX	ULTRA or ULTRA PLUS ^a
Deparaffinization	Selected	Selected
Cell Conditioning (Antigen Unmasking)	CC1, Short	ULTRA CC1, Short
Antibody (Primary)	8 minutes, 37°C	8 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."¹⁷

NEGATIVE REAGENT CONTROL

In addition to staining with CONFIRM anti-Melanosome (HMB45) 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.

Previously characterized HMB45 positive melanoma is the recommended positive control for CONFIRM anti-Melanosome (HMB45) antibody (as depicted in the image above). Cutaneous activated melanocytes often present in skin are also useful controls.

STAINING INTERPRETATION / EXPECTED RESULTS

The cellular staining pattern for CONFIRM anti-Melanosome (HMB45) antibody is cytoplasmic.

SPECIFIC LIMITATIONS

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

Sensitivity and Specificity

Table 3. Sensitivity/Specificity of CONFIRM anti-Melanosome (HMB45) antibody was determined by testing FFPE normal tissues.

Tissue	# positive / total cases	Tissue	# positive / total cases
Cerebrum	0/3	Stomach	0/3
Cerebellum	0/3	Small intestine	0/3
Adrenal gland	0/3	Colon	0/3
Ovary	0/3	Liver	0/3
Pancreas	0/3	Salivary gland	0/3
Parathyroid gland	0/3	Kidney	0/3
Pituitary gland	0/3	Prostate	0/3
Testis	0/3	Bladder	0/3
Thyroid	0/3	Endometrium	0/3
Breast	0/3	Placenta	0/3
Spleen	0/3	Uterus	0/6
Tonsil	0/3	Cervix	0/4
Thymus	0/3	Skeletal muscle	0/6
Bone marrow	0/3	Smooth muscle	0/3
Lung	0/3	Skin	12/12
Heart	0/3	Nerve	0/6
Esophagus	0/3	Mesothelium	0/6

Table 4. Sensitivity/Specificity of CONFIRM anti-Melanosome (HMB45) antibody was determined by testing a variety of FFPE neoplastic tissues.

Pathology	# positive / total cases
Glioblastoma (Cerebrum)	0/4
Pheochromocytoma (Adrenal gland)	0/1
Endometrioid adenocarcinoma (Ovary)	0/1
Mucinous adenocarcinoma (Ovary)	0/1
Ductal adenocarcinoma (Pancreas)	0/1
Neuroendocrine neoplasm (Pancreas)	0/1
Embryonal rhabdomyosarcoma (Head and neck)	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
Invasive carcinoma of no special type (Breast)	0/2
Osteosarcoma (Bone)	0/1
Adenosquamous carcinoma (Lung)	0/1
Small cell carcinoma (Lung)	0/2
Adenocarcinoma (Esophagus)	0/1
Squamous cell carcinoma (Esophagus)	0/1
Adenocarcinoma (Stomach)	0/1

Pathology	# positive / total cases
Adenocarcinoma (Small intestine)	0/1
Gastrointestinal stromal tumors, GIST (Small intestine)	0/1
Adenocarcinoma (Colon)	0/1
Gastrointestinal stromal tumors, GIST (Colon)	0/1
Hepatoblastoma (Liver)	0/1
Hepatocellular carcinoma (Liver)	0/1
Angiomyolipoma (Kidney)	20/20
Clear cell carcinoma (Kidney)	0/1
Adenocarcinoma (Prostate)	0/2
Adenocarcinoma (Rectum)	0/1
Gastrointestinal stromal tumors, GIST (Rectum)	0/1
Invasive low grade urothelial carcinoma (Bladder)	0/1
Leiomyosarcoma (Bladder)	0/1
Clear cell carcinoma (Uterus)	0/1
Endometrioid adenocarcinoma (Uterus)	0/1
Epithelioid leiomyosarcoma (Uterus)	1/1
Squamous cell carcinoma (Cervix)	0/2
Spindle cell rhabdomyosarcoma (Pelvic cavity)	0/1
Leiomyosarcoma (Broad ligament)	0/1
Neurofibroma (Soft tissue)	0/1
Basal cell carcinoma (Skin)	0/10
Squamous cell carcinoma (Skin)	0/9
Nevus (Skin)	6/7
Intradermal nevus (Skin)	1/1
Compound nevus (Skin)	2/2
Melanoma ^a	55/56
Metastatic melanoma ^b	55/56
Neuroblastoma (Retroperitoneum)	0/1
Mesothelioma (Pericardium)	0/1
Anaplastic large cell lymphoma	0/1
B-cell lymphoma, NOS	0/3
Hodgkin Lymphoma	0/1

^aIncludes skin, rectum, chest wall, vulva, eye, anus, testis, vagina tissue.

^bIncludes lymph node, liver, mediastinum, brain, parotid, soft tissue, and ear tissue

Precision

Precision studies for CONFIRM anti-Melanosome (HMB45) antibody were completed to demonstrate:

- Between lot precision of the antibody.
- Within run and between day precision on a BenchMark ULTRA instrument.
- Between instrument precision on the BenchMark GX, BenchMark ULTRA / BenchMark ULTRA PLUS instrument.
- Between platform precision between the BenchMark GX, BenchMark ULTRA / BenchMark ULTRA PLUS instrument.

All studies met their acceptance criteria.

Precision on the BenchMark ULTRA PLUS instrument was demonstrated using representative assays. Studies included 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 CONFIRM anti-Melanosome (HMB45) antibody were assessed by systematic review of the literature. The data gathered support the use of the device in accordance with its intended purpose.

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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).



Global Trade Item Number

Rx only

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

REVISION HISTORY

Rev	Updates
F	Updates to Summary and Explanation, Material Provided, Materials Required but not Provided, Warnings and Precautions, Positive Tissue Control, Analytical Performance and References sections. Updated to current template.

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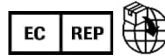
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