

Melanoma; Clone HMB45

Catalog Number	Format	Volume
A00019-0002	(Ready-To-Use)	2 ml
A00019-0007	(Ready-To-Use)	7 ml
A00019-0025	(Ready-To-Use)	25 ml
A00019-C	(Concentrate)	1 ml

Intended Use

For In-Vitro Diagnostic Use. This antibody is intended for the qualitative visualization of the anatomical elements listed in the Specificity section. It is intended to be used within an Immunohistochemistry (IHC) procedure on formalin-fixed paraffin-embedded (FFPE) human tissue followed by visualization by light microscopy.

Description

Titer/Working Dilution: Ready-to-Use: No further dilution required.
Concentrate: Suggested dilution is 1:250-500

Species: Mouse
Immunogen: BACL/C mice were injected with extract of pigmented melanoma metastases from lymph nodes.
Clone: HMB45
Isotype: Mouse IgG1 / Kappa
Format: Ready-To-Use antibody has been pre-titrated and quality controlled to work on formalin-fixed paraffin-embedded as well as acetone fixed cryostat tissue sections. No further titration is required.

Concentrate antibody is in a phosphate buffer saline containing 1% BSA.
Specificity: HMB45 has been shown to be a very specific marker for melanomas.

Background: HMB45 is a mouse monoclonal antibody that reacts against an antigen present in melanocytic tumors such as melanomas. It reacts positively against melanocytic tumors but not other tumors, thus signifying specificity and sensitivity. HMB45 is generally thought of as a melanoma specific antibody; however, there are certain exceptions. The antibody also reacts positively against junctional nevus cells but not intra-dermal nevi and against fetal melanocytes but not normal adult melanocytes. The expression of the HMB45 antigen indicates active melanosome formation and thus melanocytic differentiation. It is also expressed in normal fetal melanocytes, but not in normal resting adult melanocytes, regardless of the degree of pigmentation. Upon activation, adult melanocytes can re-express the HMB45-defined antigen (as it is expressed in fetal melanocytes). Such melanocytes are activated by a variety of stimuli. For example, HMB45 positive cells have been detected in tissue overlying or adjacent to granulation tissue, hemangiomas, vessel-rich tumor stroma, and basal cell carcinoma. Hair follicles stain occasionally due to associated stimulated melanocytes. Positive HMB45 staining has not been observed with melanocytes in lentigines or overlying fibroblastic proliferations such as keloids, dermatofibromas and old fibrotic hemangiomas. Non-melanocytic normal tissues do not react with the HMB45 antibody.

Species Reactivity: Human.

Positive Control: Human Melanoma

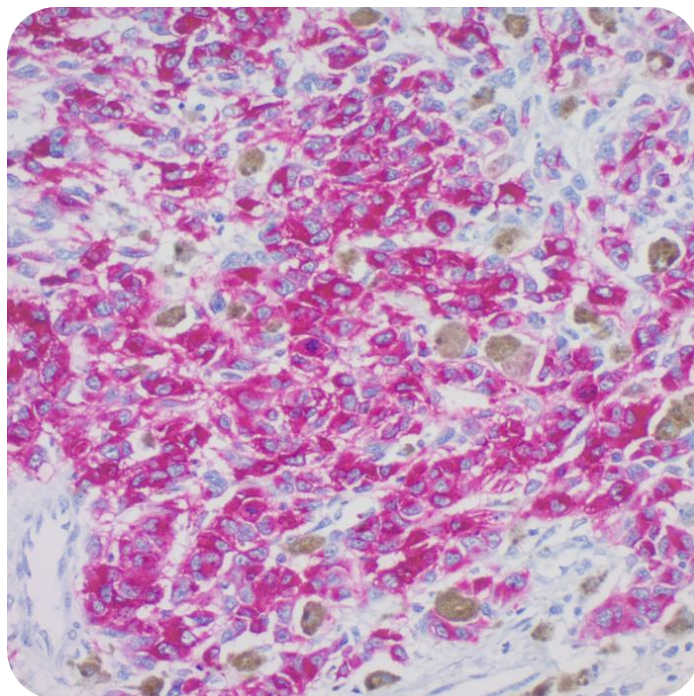
Cellular Localization: Cytoplasmic

Microbiological State: Nonsterile

Materials and Reagents Required but not Provided

1. Control tissue and reagents

2. Xylene, graded alcohols, and deionized/distilled water
 3. Antibody Diluent.
 4. IHC detection system. Suggested: ScyTek Cat# ABZ125 "CRF Anti-Polyvalent HRP Polymer" and ScyTek Cat# ACV500 "DAB Chromogen/Substrate Kit (High Contrast)".
 5. Wash buffer for rinses (ScyTek Cat# TBT500)
 6. HIER Retrieval Solution
 7. Hematoxylin counterstain and bluing reagent (ScyTek Cat# HMM500 and BRT500)
 8. Mounting medium and coverslips
- Note:** ScyTek Laboratories has a wide range of IHC reagents and ancillaries that can be found at scytex.com.



Human Melanoma stained with Ultra-Tek Alk-Phos and Permanent Red Chromogen.

Procedure

1. **Tissue Section Pretreatment (Highly Recommended):** Staining of formalin fixed paraffin embedded tissue sections is significantly enhanced by pretreatment with Tis-EDTA HIER Solution (10x) pH 9.0 (ScyTek catalog# TES500) or Citrate Plus (10x) HIER Solution (ScyTek catalog# CPL500)
2. **Primary Antibody Incubation Time:** We suggest an incubation period of 30 minutes at room temperature. However, depending upon the fixation conditions and the staining system employed, optimal incubation should be determined by the user.
3. **Visualization:** For maximum staining intensity we recommend the "UltraTek HRP Anti-Polyvalent Lab Pack" (ScyTek catalog# UHP125, see IFU for instructions) combined with the "DAB Chromogen/Substrate Bulk Pack (High Contrast)" (ScyTek catalog# ACV500, see IFU for instructions).

Storage and Stability

Storage: 2° C



8° C



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EC REP

Emergo Europe
Westervoortsedijk 60
6827 AT Arnhem, The Netherlands

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Do not Freeze. Store at 2-8°C. Return to 2-8° immediately after use. Do not use after expiration date printed on label. Verify visually that antibody has not been contaminated before use. Do not use if reagent becomes cloudy or precipitates.

Limitations

Immunohistochemistry is a complex technique involving both histological and immunological detection methods. Tissue processing and handling prior to immunostaining can cause inconsistent results. Variations in fixation and embedding or the inherent nature of the tissue specimen may cause variations in results. Endogenous peroxidase activity or pseudoperoxidase activity in erythrocytes and endogenous biotin may cause non-specific staining depending on detection system used. This data sheet's recommendations and procedures were validated using ScyTek IHC reagents and may not be suitable for other detection systems.

Precautions

1. Contains Sodium Azide as a preservative (0.09% w/v), do not ingest. 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. This product contains no hazardous material at a reportable concentration according to U.S. 29 CFR 1910.1200, OSHA Hazardous Communication Standard and EC Directive 91/155/EC.
2. Do not pipette by mouth.
3. Avoid contact of reagents and specimens with skin and mucous membranes.
4. Avoid microbial contamination of reagents or increased nonspecific staining may occur.
5. The user must validate any procedures and recommendations that differ from this data sheet.
6. The SDS may be found at scytex.com

References

1. Skelton HG, Smith KJ, Barrett TL, Lupton GP, Graham JH. HMB-45 staining in benign and malignant melanocytic lesions. *Amer J Dermatopathol* 1991;13(6):543-50.
2. Kapur RP, Bigler SA, Skelly M, Gown AM. Anti-melanoma monoclonal antibody HMB45 identifies an oncofetal glycoconjugate associated with immature melanosomes. *J Histochem Cytochem* 1992;40(2):207-12.
3. Esclamado RM, Gown AM, Vogel AM. Unique proteins defined by monoclonal antibodies specific for human melanoma. *Amer J Surg* 1986;152(4):376-85.
4. Gown AM, Vogel AM. Monoclonal antibodies to intermediate filament proteins of human cells: Unique and cross-reacting antibodies. *J Cell Biol.* 1982;95(2 Pt 1):414-24.
5. Colombari R, Bonetti F, Zamboni G, Scarpa A, Marino F, Tomezzoli A, Capelli P, Menestrina F, Chilosi M, Fiore-Donati L. Distribution of melanoma specific antibody (HMB-45) in benign and malignant melanocytic tumors. *Virch Arch A Pathol Anat* 1988;413(1):17-24.

Warranty

No products or "Instructions For Use (IFU)" are to be construed as a recommendation for use in violation of any patents. We make no representations, warranties or assurances as to the accuracy or completeness of information provided on our IFU or website. Our warranty is limited to the actual price paid for the product. ScyTek Laboratories, Inc. is not liable for any property damage, personal injury, time or effort or economic loss caused by our products.

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8° C



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