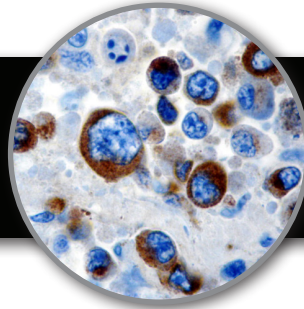


Melanosome/HMB-45

Clone: HMB-45
Mouse Monoclonal

RUO



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Inset: IHC of Melanosome/HMB-45 on a FFPE Malignant Melanoma Tissue

Intended Use

For Research Use Only.

This antibody is intended for use in Immunohistochemical applications on formalin-fixed paraffin-embedded tissues (FFPE), frozen tissue sections and cell preparations. Interpretation of results should be performed by a qualified medical professional.

Immunogen

Purified pigmented melanoma metastases from lymph nodes (HMB45).

Summary and Explanation

HMB-45 reacts against an antigen present in immature melanosomes, cutaneous melanocytes, prenatal and infantile retinal pigment epithelium and melanoma cells. This antibody was generated to an extract of Melanoma. It reacted positively against Melanocytic Tumors but not other tumors, thus demonstrating specificity and sensitivity. Moreover, this antibody reacts positively against junctional nevus cells but not intradermal nevi, and against fetal melanocytes but not normal adult melanocytes.

This antibody is very useful to identify Malignant Melanoma. Metastatic Amelanotic Melanoma can often be confused with a variety of poorly differentiated Carcinomas, Large Cell Lymphomas, Sarcomas, Spindle Cell Carcinomas and various types of mesenchymal neoplasms. A keratin-negative, vimentin-rich neoplasm that immunoreacts with antibody to S-100 protein and with this melanoma antibody, is, with rare exception, a Melanoma.

Antibody Type	Mouse Monoclonal	Clone	HMB-45
Isotype	IgG1/K	Reactivity	Paraffin, Frozen
Localization	Cytoplasmic	Control	Melanoma
Species Reactivity	Human		

Presentation

HMB-45 Melanoma antibody is a mouse monoclonal from tissue culture supernatant diluted in Phosphate Buffered Saline, pH 7.6, with protein base, and preserved with Sodium Azide preservative.

Presentations

Catalog Num.	Antibody Type	Dilution	Volume/Qty
BSB 5757	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 5758	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 5759	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 5760	Concentrated	1:250 - 1:1000	0.1 mL
BSB 5761	Concentrated	1:250 - 1:1000	0.5 mL
BSB 5762	Concentrated	1:250 - 1:1000	1.0 mL
BSB 5763	Control Slides	Not Applicable	5 slides

Precautions

1. For professional users only. Ensure results are interpreted by a medical professional.
2. This product contains sodium azide (NaN₃), a toxic chemical which may react with plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent sodium azide build-up.
3. Ensure proper handling procedures are used with reagent. Always wear proper laboratory equipment such as laboratory coat and gloves when handling reagents.
4. Unused solution should be disposed of according to local and federal regulations.
5. Do not ingest reagent. If reagent ingested, contact a poison control center immediately.
6. For complete recommendations for handling biological specimens please refer to the CDC document, "Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories" (4).

Storage

Store at 2-8 °C. Do not use after expiration date listed on package label. Temperature fluctuations should be avoided. Store appropriately when not in use, and avoid prolonged exposure to room temperature conditions.

Specimen Preparation

Paraffin sections: The antibody can be used on formalin-fixed paraffin-embedded (FFPE) tissue sections. Ensure tissue undergoes appropriate fixation to ensure best results. Pre-treatment of tissues with heat-induced epitope retrieval (HIER) is recommended using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023), ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033) or ImmunoDNA Digestor (BSB 0108-0112). See reverse side for complete protocol. Tissue should remain hydrated via use of Bio SB Immuno/DNA Washer solutions (BSB 0029 & BSB 0042).

Frozen sections and cell preparations: The antibody can be used for labeling acetone-fixed frozen sections and acetone-fixed cell preparations.

Staining Procedure

1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positive charged slides such as Bio SB Hydrophilic Plus Slides (BSB 7028).
2. Air dry for 2 hours at 58° C.
3. Deparaffinize, dehydrate and rehydrate tissues.
4. Subject tissues to heat epitope retrieval using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or EDTA (BSB 0030-BSB 0033).
5. Any of three heating methods may be used:

a. TintoRetriever Pressure Cooker or Equivalent

Place tissues/slides in a staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA, and place in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high. Incubate for 15 minutes. Open and immediately transfer slides to room temperature.

b. TintoRetriever PT Module or Water Bath Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA at 95°-99° C. Incubate for 30-60 minutes.

c. Conventional Steamer Method

Place tissues/slides in a pre-warmed staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate or EDTA in a Steamer, cover and steam for 30-60 minutes.

6. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
7. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
8. Wash slides with IHC wash buffer or DI water.
9. Continue IHC staining protocol.

Product Limitations

Due to inherent variability present in immunohistochemical procedures (including fixation time of tissues, dilution factor of antibody, retrieval method utilized and incubation time), optimal performance should be established through the use of positive and negative controls. Results should be interpreted by a medical professional.





References

1. Gown AM, et al. A J Path. 1986;123:195
2. Wick MR, et al. Arch Path Lab. 1988;112:616
3. Leong ASY, et al. Surg Path. 1989;2:137
4. U.S. Department of Health and Human Services: Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. Supplement / Vol. 61, January 6, 2012.

Recommended IHC Protocol

Step	ImmunoDetector AP/HRP	PolyDetector AP/HRP	PolyDetector Plus HRP
Peroxidase/AP Blocker	5 min.	5 min.	5 min
Primary Antibody	30-60 min.	30-60 min.	30-60 min.
1st Step Detection	10 min.	30-45 min.	15 min.
2nd Step Detection	10 min.	Not Applicable	15 min.
Substrate-Chromogen	5-10 min.	5-10 min.	5-10 min.
Counterstain	Varies	Varies	Varies

Symbol Key / Légende des symboles/Erläuterung der Symbole

 8°C 2°C	Storage Temperature Limites de température Zulässiger temperaturbereich		Manufacturer Fabricant Hersteller	REF	Catalog Number Référence du catalogue Bestellnummer
	Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten		Expiration Date Utiliser jusque Verwendbar bis	LOT	Lot Number Code du lot Chargenbezeichnung

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