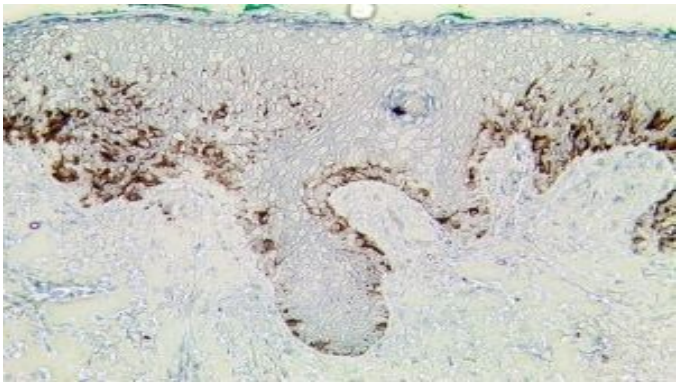
TintoFast Melanoma Cocktail: HMB-45, Mart-1 & Tyrosinase

CE IVD

Clone: HMB-45, A103 & BSB-6

Mouse Monoclonal



Inset: IHC of TintoFast Melanoma Cocktail: HMB-45, Mart-1 & Tyrosinase on a FFPE Lentigo Maligna Carcinoma Tissue

Intended Use

For Mohs In Vitro Diagnostic Use.

This antibody is intended for the fast immunohistochemical detection of melanomas and melanocytic lesions during intraoperative Mohs surgery on frozen sections. Additionally, this antibody can also be used on FFPE specimens. Interpretation of results should be performed by a qualified medical professional.

Immunogen

Pigmented melanoma metastases from lymph nodes (HMB45); Recombinant human Melan-A protein (A103) and Recombinant full length human tyrosinase protein (BSB6).

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 is very useful to identify Malignant Melanoma. MART-1/ Melan-A is a protein antigen found on melanocytes. Antibodies against this antigen are used to recognize cells of melanocytic differentiation, useful for the diagnosis of Melanoma. The same name is used to refer to the gene which codes for this antigen. Tyrosinase is a copper-containing enzyme present in plant and animal tissues that catalyzes the production of melanin and other pigments from tyrosine by oxidation.

The MART-1/Melan-A antigen is specific for the melanocyte lineage found in normal skin, retina, and melanocytes, but not in other normal tissues. It is thus useful as a marker for Melanocytic Tumors, with the caveat that it is normally found in benign nevi as well. Anti-Tyrosinase has been found to be quite specific for melanocytic lesions such as Malignant Melanoma and Melanotic Neurofibroma. Essentially no carcinomas express this marker. Melanoma cocktail HMB-45, Mart-1 and Tyrosinase are ideally suited to detect melanomas and melanocytic lesions and may prove to be a valuable marker for melanoma metastasis in sentinel lymph nodes.

Antibody Type	Mouse Monoclonal	**Clone**	HMB-45, A103 & BSB-6
Isotype	IgG1/K, IgG1 & IgG2a	**Reactivity**	Paraffin, Frozen
Localization	Cytoplasmic	**Control**	Skin, Melanoma
	Species Reactivity	Human, Dog	

Presentation

Anti-TintoFast Melanoma Cocktail: HMB-45, Mart-1 & Tyrosinase is a Mouse Monoclonal antibody derived from cell culture supernatant that is concentrated, dialyzed, filter sterilized and diluted in buffer pH 7.5, containing BSA and sodium azide as a preservative.

Catalog No.	*Antibody Type*	*Dilution*	*Volume/Qty*
BSB-3695-3	TintoFast Predilute	Ready-to-Use	3.0 mL
BSB-3695-7	TintoFast Predilute	Ready-to-Use	7.0 mL
BSB-3695-15	TintoFast Predilute	Ready-to-Use	15.0 mL

Control Slides Available

Catalog No.	*Quantity*
BSB-3695-CS	5 slides

Storage Store at 2-8°C (Control Slides: Store at 20-25°C)

Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains <0.1% sodium azide (NaN_3) as a preservative. Ensure proper handling procedures are used with this reagent.
3. Always wear personal protective equipment such as a laboratory coat, goggles, and gloves when handling reagents.
4. Dispose of unused solution with copious amounts of water.
5. Do not ingest reagent. If reagent is ingested, seek medical advice immediately.
6. Avoid contact with eyes. If contact occurs, flush with large quantities of water.
7. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker (BSB 7008), TintoDetector Incubator (BSB 7002) or similar.
8. For additional safety information refer to Safety Data Sheet for this product.
9. 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" (see References in this document).

Stability

This product is stable up to the expiration date on the product label.

Do not use after expiration date listed on the package label. Temperature fluctuations should be avoided. Store appropriately when not in use and avoid prolonged exposure to room temperature conditions.

Mohs IHC Protocol

Specimen Preparation of Mohs Frozen Tissues

1. Embed the specimen in OCT inside a cryostat.
2. Cut sections at 4-5 µm and mount on a positively charged glass slide such as the Bio SB Hydrophilic Plus Slides (BSB 7028) or TintoDetector Cap Gap slides (BSB 7006).
3. Air dry the slide at room temperature for 2 minutes and then incubate the slide at 60 °C for 3 minutes in an incubator or dry bath.
4. Fix in 100% acetone or 10% NBF for 2 minutes at room temperature. Choice of fixation solution depends on antibody.
5. If tissue is fixed in 100% acetone, let the slide air dry. If the tissue is fixed in 10% NBF, rinse with distilled water and then dry dry the slides for 2 minutes at room temperature.

Tissue Pretreatment Procedure for Mohs Frozen Tissues

1. Subject tissues to HIER (heat-induced epitope retrieval) using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023). Optionally, Mohs Immuno Digestor (BSB 0108-0112) can be used for cytokeratin targets instead of HIER.

a. For Mohs PolyDetector HRP Green or DAB protocol use the TintoRetriever Pressure Cooker (BSB 7008) or Equivalent. Place tissues/slides in a staining dish or coplin jar containing the ImmunoDNA Retriever with Citrate , and place on trivet or staining dish support in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high (110-121° C). Incubate for 5 minutes. Open and immediately transfer slides to room temperature. Cool off for 5 -10 min

b. For Mohs PolyDetector Plus HRP Green or DAB protocol use the TintoDetector Incubator (BSB 7002). Preheat the TintoDetector Incubator to 110 °C. Place TintoDetector Cap Gap slides (BSB 7006) face to face and insert them into the TintoDetector Slide Holder (BSB 7003). Submerge slides in ImmunoDNA Retriever with Citrate to draw up enough solution by capillary action to cover the tissues. Heat the slides in a preheated TintoDetector Incubator for 3 minutes. Transfer slides to room temperature and cool off for 1 min.

c. For targets that are compatible with Mohs Immuno Digestor, Incubate with Mohs ImmunoDigestor at room temperature for 1 min and rinse the slides with ImmunoDNA washer (BSB 0029 & BSB 0042) after 1 min.

Mohs IHC Detection

1. After HIER, transfer slides to ImmunoDNA washer and let it stand for 1-2 minutes.
2. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
3. Wash slides with ImmunoDNA washer or DI water.
4. Continue IHC detection protocol. Wash slides between each step with ImmunoDNA washer solution.

Abbreviated Mohs Immunohistochemical Protocol

Step	Mohs PolyDetector HRP Green or DAB 10 min* Protocol	Mohs PolyDetecto Plus HRP Green or DAB 20 min Protocol
HIER	5 min**	3 min
Primary Antibody	4 min.	5 min.
1st Step Detection	3 min.	4 mn.
2nd Step Detection	NA.	4 min.
Substrate-Chromogen	2 min.	1-2 min.
Counterstain / Coverslip	Varies	Varies

*instrument setup and HIER time not included
**1 min PIER for compatible targets

IHC Protocol for FFPE Tissues

1. Cut and mount 3-5 micron formalin-fixed paraffin-embedded tissues on positively charged slides such as Bio SB Hydrophilic Plus Slides.
2. Air dry for 2 hours at 58° C.

Tissue Pretreatment Procedure for FFPE Tissues

1. Deparaffinize, dehydrate, and rehydrate tissues.
2. Subject tissues to HIER using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate or EDTA.
3. 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 on trivet in the pressure cooker. Add 1-2 inches of distilled water to the pressure cooker and turn heat to high. Incubate for 10- 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 20-30 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 20-30 minutes.

4. After HIER, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 10 minutes.
5. For manual IHC, perform antibody incubation at ambient temperature. For automated IHC methods, perform antibody incubation according to instrument manufacturer's instructions.
6. Wash slides with ImmunoDNA washer or DI water.
7. Continue IHC protocol. Wash slides between each step with ImmunoDNA washer solution.

Mounting Protocols

Mount with aqueous media such as AquaMounter (BSB-0090- BSB 0093) or permanent mounting media such as XyGreen PermaMounter (BSB 0169-0174) or organic solvent-based resin such as PermaMounter (BSB 0094-0097).

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 qualified medical professional.

References

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Symbol Key / Légende des symboles/Erläuterung der Symbole

EC REP — EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands	Storage Temperature / Limites de température / Zulässiger Temperaturbereich	Manufacturer / Fabricant / Hersteller	REF — Catalog Number / Référence du catalogue / Bestellnummer
IVD — In Vitro Diagnostic Medical Device / Dispositif médical de diagnostic in vitro / In-Vitro-Diagnostikum	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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