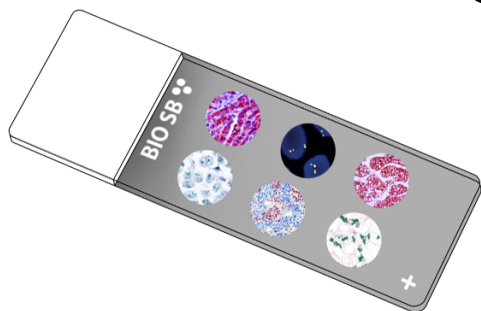


# Melanoma Cocktail: HMB-45, MART-1 & Tyrosinase Control Slides



## Intended Use

For In Vitro Diagnostic Use.

## 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.

## Presentation

Five slides of Melanoma Cocktail: HMB-45, MART-1 & Tyrosinase positive tissues, each mounted on Hydrophilic Plus Slides, provided in a plastic mailer.

| <i>Catalog No.</i> | <i>Quantity</i> |
|--------------------|-----------------|
| BSB-9273-CS        | 5 slides        |
| BSB 6883           | 5 slides        |

**Storage** Store at 20-25°C

## Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. 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. Follow safety precautions of the heating device used for epitope retrieval (TintoRetriever Pressure Cooker 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 package label.

## IHC Protocol

1. Subject tissues to heat induced epitope retrieval (HIER) using a suitable retrieval solution such as ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or EDTA (BSB 0030-BSB 0033).

2. 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 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.

3. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.
4. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.
5. Wash slides with ImmunoDNA washer or DI water.
6. Continue IHC staining protocol. Wash slides between each step with ImmunoDNA washer solution.

### Abbreviated Immunohistochemical Protocol

| Step                     | ImmunoDetector<br>AP/HRP | PolyDetector<br>AP/HRP | PolyDetector<br>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 / Coverslip | Varies                   | Varies                 | Varies                   |

### Abbreviated IF Protocol

| Step                                           | Incubation Time   |
|------------------------------------------------|-------------------|
| Rinse slides in IF wash buffer                 | 5 minutes         |
| Drain and wipe excess IF wash buffer off slide |                   |
| Conduct remaining steps in the dark            |                   |
| Apply Antibody                                 | 30-60 minutes     |
| Rinse with 3 changes of IF wash buffer         | 3x15 minutes each |
| Coverslip with IF mounting medium              |                   |

### Mounting Protocols

For detailed instructions using biodegradable permanent mounting media such as XyGreen PermaMunter (BSB 0169-0174) or organic solvent based resin such as PermaMunter (BSB 0094-0097), refer to PI0174 or PI0097.

### 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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4. Orsz Z, Histopathology. 1999;Jun;34(6):517-25
5. Kaufmann O, et al. Mod Pathol. 1998;Aug;11(8):740-6
6. Busam KJ, et al. Am J Dermatopathol. 2000;June;22(3):237-41
7. Jungbluth AA, et al. Pathol Res Pract. 2000;196(4):235-42
8. Meije CB, et al. J Pathol. 2000;Apr;190(5):572-8
9. 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. <https://www.cdc.gov/mmwr/pdf/other/su6101.pdf>

### Symbol Key / Légende des symboles/Erläuterung der Symbole

|                      |                                                                                                          |                                                                                                                                                                                             |                                                                                                                                          |            |                                                           |
|----------------------|----------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------|------------|-----------------------------------------------------------|
| <b>EC</b> <b>REP</b> | QAdvis EAR AB<br>Ideon Science Park<br>Scheelevägen 17<br>SE-223 70 Lund, Sweden                         |  Storage Temperature<br>Limites de température<br>Zulässiger Temperaturbereich                           |  Manufacturer<br>Fabricant<br>Hersteller              | <b>REF</b> | Catalog Number<br>Référence du catalogue<br>Bestellnummer |
| <b>IVD</b>           | In Vitro Diagnostic Medical Device<br>Dispositif médical de diagnostic in vitro<br>In-Vitro-Diagnostikum |  Read Instructions for Use<br>Consulter les instructions<br>d'utilisation<br>Gebrauchsanweisung beachten |  Expiration Date<br>Utiliser jusque<br>Verwendbar bis | <b>LOT</b> | Lot Number<br>Code du lot<br>Chargenbezeichnung           |