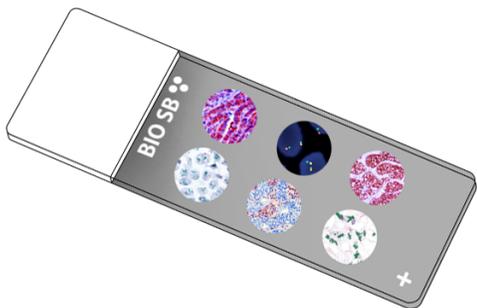


# PRAME Control Slides



### Intended Use

For In Vitro Diagnostic Use.

### Summary and Explanation

Melanoma antigen preferentially expressed in tumors is a protein that in humans is encoded by the PRAME gene. This gene encodes an antigen that is predominantly expressed in human melanomas and that is recognized by cytolytic T lymphocytes. It is not expressed in normal tissues, except in testis. This expression pattern is like that of other CT antigens, such as MAGE, BAGE, and GAGE. However, unlike these other CT antigens, this gene is also expressed in acute leukemias. PRAME overexpression in triple negative breast cancer has also been found to promote cancer cell motility through induction of the epithelial-to-mesenchymal transition. PRAME mRNA expression is well documented in cutaneous and ocular melanomas. One study concluded that diffuse nuclear immunoreactivity for PRAME was found in 87% of metastatic and 83.2% of primary melanomas. Among melanoma subtypes, PRAME was diffusely expressed in 94.4% of acral melanomas, 92.5% of superficial spreading melanomas, 90% of nodular melanomas, 88.6% of lentigo maligna melanomas, and 35% of desmoplastic melanomas. When in situ and non-desmoplastic invasive melanoma components were present, PRAME expression was seen in both. Most Melanocytic nevi (86.4%), were completely negative for PRAME. Immunoreactivity for PRAME was seen, albeit usually only in a minor subpopulation of lesional melanocytes, in 13.6% of cutaneous nevi, including dysplastic nevi, common acquired nevi, traumatized/recurrent nevi, and Spitz nevi. Rare isolated junctional melanocytes with immunoreactivity for PRAME were also seen in solar lentigines and benign nonlesional skin. This study suggests that immunohistochemical analysis for PRAME expression may be useful for diagnostic purposes to support a suspected diagnosis of melanoma. It may also be valuable for margin assessment of a known PRAME-positive melanoma, but its expression in nevi, solar lentigines, and benign nonlesional skin can represent a challenge.

### Presentation

Five slides of PRAME positive tissues, each mounted on Hydrophilic Plus Slides, provided in a plastic mailer.

| <i>Catalog No.</i> | <i>Quantity</i> |
|--------------------|-----------------|
| BSB-9351-CS        | 5 slides        |
| BSB-2374-CS        | 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 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 / 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

1. "Entrez Gene: PRAME preferentially expressed antigen in melanoma": <https://www.ncbi.nlm.nih.gov/gene?Db=gene&Cmd=ShowDetailView&TermToSearch=23532>
2. Al-Khadairi G, et al. "PRAME promotes epithelial-to-mesenchymal transition in triple negative breast cancer". Journal of Translational Medicine. 2019; 17 (1): 9
3. Lezcano C, et al. PRAME Expression in Melanocytic Tumors. Am J Surg Pathol. 2018 Nov; 42(11): 1456-1465
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.

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

|               |  |   |  |            |   |
|---------------|--|---|--|------------|---|
| <b>EC 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           |