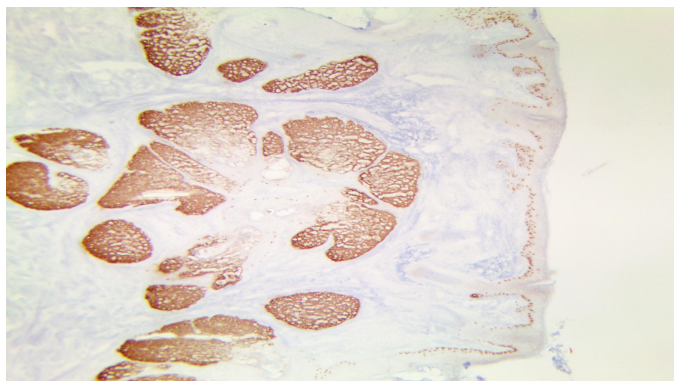


TintoFast PRAME

Clone: RBT-PRAME
Rabbit Monoclonal



Inset: IHC of PRAME on a Frozen Melanoma Tissue

Intended Use

For Mohs In Vitro Diagnostic Use.

This antibody is intended for the fast immunohistochemical detection of PRAME expressed in melanomas 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

Synthetic peptide corresponding to residues of human PRAME protein.

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 is recognized by cytolytic T lymphocytes. PRAME 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 antigen 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 NonDesmoplastic 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.

Antibody Type	Rabbit Monoclonal	Clone	RBT-PRAME
Isotype	IgG	Reactivity	Paraffin, Frozen
Localization	Nuclear	Species Reactivity	Human
Control	Skin		
Application	Mohs, Melanoma & Skin Cancer		

Presentation

Anti-TintoFast PRAME is a Rabbit 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.

<i>Catalog No.</i>	<i>Presentation</i>	<i>Dilution</i>	<i>Volume</i>
BSB-3774-3	TintoFast Predilute	Ready-to-Use	3.0 mL
BSB-3774-7	TintoFast Predilute	Ready-to-Use	7.0 mL
BSB-3774-15	TintoFast Predilute	Ready-to-Use	15.0 mL

Precautions

1. For professional users only. Results should be interpreted by a qualified medical professional.
2. This product contains <0.1% sodium azide (NaN₃) 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 for 2 minutes at room temperature and let the slide air dry.

Tissue Pretreatment Procedure for Mohs Frozen Tissues

Subject tissues to HIER (heat-induced epitope retrieval) using Bio SB ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033).

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 EDTA 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.

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

*instrument setup and HIER time not included

IHC Protocol for FFPE Tissues

Specimen Preparation of 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.

IHC Detection

1. After HIER, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 10 minutes.
2. For manual IHC, perform antibody incubation at ambient temperature. For automated IHC methods, perform antibody incubation according to instrument manufacturer's instructions.
3. Wash slides with ImmunoDNA washer or DI water.
4. 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 apply Fast ChromoProtector (BSB 0327) and then 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

1. "Entrez Gene: 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

	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands	 Storage Temperature Limites de température Zulässiger Temperaturbereich	 Manufacturer Fabricant Hersteller	 Catalog Number Référence du catalogue Bestellnummer
	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 Number Code du lot Chargenbezeichnung