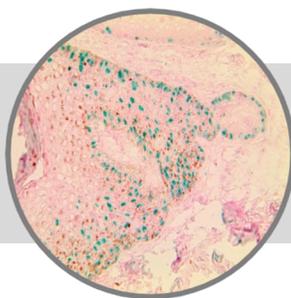


TintoFast MiTF

Clone: C5/D5

Mouse Monoclonal

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Inset: IHC of MiTF on a Frozen Acetone-Fixed Melanoma Tissue

Intended Use

For Research Use Only.

This antibody is intended for the fast immunohistochemical detection of melanocytes or melanoma cells 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

N-terminal fragment of the human MiTF protein.

Summary and Explanation

Microphthalmia-associated Transcription Factor (MiTF) is a basic helix-loop-helix leucine zipper transcription factor involved in melanocyte and osteoclast development. Mutations in MiTF cause auditory pigmentary syndromes, such as Waardenburg Syndrome Type II, Type IIa and Tietz Syndrome in humans. There are two known isoforms of MiTF differing by 66 amino acids at the NH2 terminus. Shorter forms are expressed in melanocytes and run as two bands at 52 kDa and 56 kDa, while the longer Mi form runs as a cluster of bands at 60-70 kDa in osteoclasts and in B16 Melanoma cells (but not other Melanoma cell lines), as well as mast cells and heart cells. MiTF plays a critical role in the differentiation of various cell types such as neural crest-derived melanocytes, mast cells, osteoclasts and optic cup-derived retinal pigment epithelium.

This antibody recognizes serine phosphorylated and non-phosphorylated melanocytic isoforms of microphthalmia. It is useful in identifying Malignant Melanoma, and distinguishing mast cell lesions from lesions of myeloid derivation. A relatively rare class of tumors known as PEComas (tumors showing perivascular epithelioid cell differentiation) express MiTF in a high percentage of cases (~90%).

Antibody Type	Mouse Monoclonal	Clone	C5/D5
Isotype	IgG1/K	Reactivity	Paraffin, Frozen
Localization	Nuclear	Control	Skin, Melanoma
Species Reactivity		Human, Dog, Cat	

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 laboratory coat, goggles and gloves when handling reagents.
4. Dispose of unused solution with copious amount 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 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).

Presentation

Anti-MiTF is cocktail of two mouse monoclonal antibodies derived from cell culture supernatant that are 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 3657	TintoFast Prediluted	Ready-to-Use	3.0 mL
BSB 3658	TintoFast Prediluted	Ready-to-Use	7.0 mL
BSB 3659	TintoFast Prediluted	Ready-to-Use	15.0 mL

Storage Store at 2-8°C

Stability

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

Mohs Frozen sections and cell preparations: The antibody can be used for the detection of melanocytes and melanoma cells in Mohs acetone-fixed frozen sections.

Paraffin sections: The antibody can be used on formalin-fixed paraffin-embedded (FFPE) tissue sections. Ensure tissue undergoes appropriate fixation for 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). Tissue should remain hydrated via use of Bio SB Immuno/DNA Washer solutions (BSB 0029 & BSB 0042).

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.

Mohs IHC Procedure

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).
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.
5. Rinse with distilled water and air dry the slides for another 2 minutes at room temperature.

Tissue Pretreatment Procedure for Mohs Frozen Tissues

1. Subject tissues to epitope retrieval using Bio SB ImmunoDNA Retriever with Citrate (BSB 0020-BSB 0023) or ImmunoDNA Retriever with EDTA (BSB 0030-BSB 0033). It is recommended that user uses a **5 minute** heat-induced epitope retrieval (HIER) method using a pressure cooker (BSB TintoRetriever Pressure Cooker) at 110°C.
2. Any of three HIER 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 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. 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 10-15 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 10-15 minutes.

IHC Detection Procedure

1. After HIER, transfer slides to ImmunoDNA washer and let 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.

References

1. Levy C, et al. Trends Mol Med. 2006;12(9):406-14
2. Liegl B, et al. Am J Surg Pathol. 2008;Apr;32(4):608-14
3. Ohsie SJ, et al. J Cutan Pathol. 2008;May;35(5):433-44
4. Smith SD, et al. J Med Genet. 2000;37(6):446-8
5. Zhuang L, et al. Mod Pathol. 2007;Apr;20(4):416-26
6. 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.

Abbreviated Mohs Immunohistochemical Protocol

For best results we recommend using Mohs Frozen sections fixed with acetone for 2 min. then HIER with Citrate for 5 min at 110-121 ° C.

Step	Mohs PolyDetector HRP Green or DAB 5 min Protocol	Mohs PolyDetector HRP Green or DAB 10 min Protocol
Peroxidase/AP Blocker	0.5 min.	0.5 min.
Primary Antibody	2 min	4 min.
1st Step Detection	1 min	3 min.
Substrate-Chromogen	1 min	2 min.
Counterstain / Coverslip	0.5 min	0.5 min.

This protocol can also be used with FFPE Tissues retrieved with Citrate or EDTA.

Specimen Preparation 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 (BSB 7028).
2. Air dry for 2 hours at 58° C.

Tissue Pretreatment Procedure for FFPE Tissues

1. Deparaffinize and rehydrate tissues.
2. 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).
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 heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 10 minutes.

5. For manual staining, perform antibody incubation at ambient temperature. For automated staining methods, perform antibody incubation according to instrument manufacturer's instructions.

6. Wash slides with ImmunoDNA washer or DI water.

7. Continue Mohs IHC staining protocol. Wash slides between each step with ImmunoDNA washer solution.

Mounting Protocols

Mount with aqueous mounting 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).

Symbol Key / Légende des symboles/Erläuterung der Symbole

	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

