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Inset: IHC of MiTF on a Melanoma Tissue

Intended Use

For In Vitro Diagnostic Use.

This antibody is intended for use in Immunohistochemical applications on formalinfixed paraffin-embedded tissues (FFPE), frozen tissue sections and cell preparations. 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		
lsotype	lgG1/K	Reactivity	Paraffin, Frozen		
Localization	n Nuclear Control Skin, Melar		Skin, Melanoma		
	Species Reactivity	Human, Dog, Cat			

Precautions

1. For professional users only. Results should be interpreted by a gualified 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 6247	Tinto Prediluted	Ready-to-Use	3.0 mL
BSB 6248	Tinto Prediluted	Ready-to-Use	7.0 mL
BSB 6249	Tinto Prediluted	Ready-to-Use	15.0 mL
BSB 6250	Concentrated	1:250 - 1:1000	0.1 mL
BSB 6251	Concentrated	1:250 - 1:1000	0.5 mL
BSB 6252	Concentrated	1:250 - 1:1000	1.0 mL

Control Slides Available

Catalog No.	Quantity		
BSB 6253	5 slides		

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

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) or ImmunoDNA Digestor (BSB 0108-0112). See reverse side for complete protocol. Tissue should remain hydrated via use of Bio SB Immuno/DNA Washer solutions (BSB 0029 & BSB 0042).

Frozen sections and cell preparations: The antibody can be used for labeling acetone-fixed frozen sections and acetone-fixed cell preparations.

Staining Procedure

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.

3. Deparaffinize, dehydrate and rehydrate tissues.

4. 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).

5. 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.

6. After heat treatment, transfer slides in ImmunoDNA Retriever with Citrate or EDTA to room temperature and let stand for 15-20 minutes.

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

8. Wash slides with ImmunoDNA washer or DI water.

9. 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	

Mounting Protocols

PI 6252, Rev. D DCN: 3566

For detailed instructions using biodegradable permanent mounting media such as XyGreen PermaMounter (BSB 0169-0174) or organic solvent based resin such as PermaMounter (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. 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, at 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.

Symbol Key / Légende des symboles/Erläuterung der Symbole

EC REF	EMERGO EUROPE Prinsessegracht 20 2514 AP The Hague The Netherlands	2 C A SC	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	[]i	Read Instructions for Use Consulter les instructions d'utilisation Gebrauchsanweisung beachten	\sum	Expiration Date Utiliser jusque Verwendbar bis	LOT	Lot Number Code du lot Chargenbezeichnung



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