

Microphthalmia Transcription Factor (MiTF)

Concentrated and Prediluted Monoclonal Antibody
901-423-060420

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M E D I C A L

Catalog Number:	CM 423 BK	PM 423 AA	AVI 423 G	VLTM 423 G20
Description:	0.5 mL, conc.	6.0 mL, RTU	6.0 mL, RTU	20 mL, RTU
Dilution:	1:25	Ready-to-use	Ready-to-use	Ready-to-use
Diluent:	Renoir Red	N/A	N/A	N/A

Intended Use:

For In Vitro Diagnostic Use

Microphthalmia Transcription Factor (MiTF) [34CA5] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of MiTF protein by immunohistochemistry (IHC) in formalin-fixed paraffin-embedded (FFPE) human tissues. The clinical interpretation of any staining or its absence should be complemented by morphological studies using proper controls and should be evaluated within the context of the patient's clinical history and other diagnostic tests by a qualified pathologist.

Summary and Explanation:

Microphthalmia transcription factor (MiTF) was recently cloned as the human homolog of the mouse microphthalmia (mi) gene product. The mi phenotype is associated with a mutant mi locus and characterized by small eyes and loss of melanin pigments. MiTF is the only nuclear melanocytic marker and is a sensitive and specific marker for malignant melanoma, including some spindle-cell variants, in cytologic specimens, and may be superior to the current standard melanocytic markers, S100 protein and HMB45 antigen. MiTF may be very valuable for the diagnosis of melanoma, including desmoplastic variants; melanocytic soft tissue tumors, such as clear cell sarcoma; and the unusual group of tumors that show combined melanocytic and myoid differentiation, the perivascular epithelioid cell family of tumors (PEComas). Microphthalmia transcription factor may be a valuable addition to the marker panel used in diagnosing melanoma, in combination with S100, HMB45, Tyrosinase and MART-1.

Principle of Procedure:

Antigen detection in tissues and cells is a multi-step immunohistochemical process. The initial step binds the primary antibody to its specific epitope. After labeling the antigen with a primary antibody, a one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

Source: Mouse monoclonal

Species Reactivity: Human; others not tested

Clone: 34CA5

Isotype: IgG1/kappa

Protein Concentration: Call for lot specific Ig concentration.

Epitope/Antigen: Human MiTF

Cellular Localization: Nuclear

Positive Tissue Control: Melanoma

Known Applications:

Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Supplied As: Buffer with protein carrier and preservative
Renoir Red (PD904)

Storage and Stability:

Store at 2°C to 8°C. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Protocol Recommendations (VALENT® Automated Slide Staining Platform):

VLTM423 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:

Note: VLTM423 requires an offline deparaffinization and pretreatment step prior to running on the VALENT (see below).

Deparaffinization: Perform offline standard deparaffinization with series of xylenes and alcohols.

Pretreatment: Perform offline heat retrieval using Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions. Rinse slides in distilled water and transfer to the VALENT. Do not let slides dry out during this step.

Peroxidase Block: Block for 5 minutes with Val Peroxidase Block.

Protein Block (Optional): Incubate for 10-20 minutes at RT with Val Background Block.

Primary Antibody: Incubate for 30 minutes.

Secondary: Incubate for 10 minutes with Val Mouse Secondary.

Linker: Incubate for 10 minutes with Val Universal Linker.

Polymer: Incubate for 10 minutes with Val Universal Polymer.

Chromogen: Incubate for 5 minutes with Val DAB.

Counterstain: Counterstain for 5 minutes with Val Hematoxylin.

Protocol Recommendations (intelliPATH FLX® and manual use):

Peroxide Block: Block for 5 minutes with Peroxidized 1.

Pretreatment: Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker data sheet for specific instructions.

Protein Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.

Primary Antibody: Incubate for 30-60 minutes at RT.

Probe: Incubate for 10 minutes at RT with a secondary probe.

Polymer: Incubate for 10-20 minutes at RT with a tertiary polymer.

Chromogen: Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Warp Red.

Counterstain:

Counterstain with hematoxylin. Rinse with deionized water. Apply Tacha's Bluing Solution for 1 minute. Rinse with deionized water.

Technical Note:

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

Protocol Recommendations (Ventana BenchMark ULTRA):

AVI423 is intended for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

Template/Detection: OptiView DAB IHC

Pretreatment Protocol: CC1 92 minutes

Peroxidase: Pre Primary Peroxidase Inhibitor

Primary Antibody: 60 minutes, 36°C

Amplification Kit: 4 minutes, 4 minutes

Limitations:

The optimum antibody dilution and protocols for a specific application can vary. These include, but are not limited to fixation, heat-retrieval method, incubation times, tissue section thickness and detection kit used. Due to the superior sensitivity of these unique reagents, the recommended incubation times and titers listed are not applicable to other detection systems, as results may vary. The data sheet recommendations and protocols are based on exclusive use of Biocare

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Limitations Cont'd:

products. Ultimately, it is the responsibility of the investigator to determine optimal conditions.

Quality Control:

Refer to CLSI Quality Standards for Design and Implementation of Immunohistochemistry Assays; Approved Guideline-Second edition (I/LA28-A2) CLSI Wayne, PA USA (www.clsi.org). 2011

Precautions:

1. This antibody contains less than 0.1% sodium azide. Concentrations less than 0.1% are not reportable hazardous materials according to U.S. 29 CFR 1910.1200, OSHA Hazard communication and EC Directive 91/155/EC. Sodium azide (NaN₃) used as a preservative is toxic if ingested. Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. Upon disposal, flush with large volumes of water to prevent azide build-up in plumbing. (Center for Disease Control, 1976, National Institute of Occupational Safety and Health, 1976) (6)
2. Specimens, before and after fixation, and all materials exposed to them should be handled as if capable of transmitting infection and disposed of with proper precautions. Never pipette reagents by mouth and avoid contacting the skin and mucous membranes with reagents and specimens. If reagents or specimens come into contact with sensitive areas, wash with copious amounts of water. (7)
3. Microbial contamination of reagents may result in an increase in nonspecific staining.
4. Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change.
5. Do not use reagent after the expiration date printed on the vial.
6. The SDS is available upon request and is located at <http://biocare.net>.

Troubleshooting:

Follow the antibody specific protocol recommendations according to data sheet provided. If atypical results occur, contact Biocare's Technical Support at 1-800-542-2002.

References:

1. Ohsie SJ, *et al.* Immunohistochemical characteristics of melanoma. J Cutan Pathol. 2008 May; 35(5):433-44.
2. Sheffield MV, *et al.* Comparison of five antibodies as markers in the diagnosis of melanoma in cytologic preparations. Am J Clin Pathol. 2002 Dec; 118(6):930-6.
3. Dorvault CC, *et al.* Microphthalmia transcription factor: a sensitive and specific marker for malignant melanoma in cytologic specimens. Cancer. 2001 Oct 25; 93 (5):337-43.
4. O'Reilly FM, *et al.* Microphthalmia transcription factor immunohistochemistry: a useful diagnostic marker in the diagnosis and detection of cutaneous melanoma, sentinel lymph node metastases, and extracutaneous melanocytic neoplasms. J Am Acad Dermatol. 2001 Sep; 45(3):414-9.
5. Miettinen M, *et al.* Microphthalmia transcription factor in the immunohistochemical diagnosis of metastatic melanoma: comparison with four other melanoma markers. Am J Surg Pathol. 2001 Feb; 25(2):205-11.
6. Center for Disease Control Manual. Guide: Safety Management, NO. CDC-22, Atlanta, GA. April 30, 1976 "Decontamination of Laboratory Sink Drains to Remove Azide Salts."
7. Clinical and Laboratory Standards Institute (CLSI). Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.

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