MART-1 + Tyrosinase + SOX10

Prediluted Monoclonal Antibody Cocktail
Control Number: 901-3165-010919

BIOCARE MEDICAL

Catalog Number:	**API 3165 AA**
Description:	6.0 mL, RTU
Dilution:	Ready-to-use
Diluent:	N/A

Intended Use:
For In Vitro Diagnostic Use
MART-1 + Tyrosinase + SOX10 is a cocktail of mouse monoclonal antibodies that is intended for laboratory use in the qualitative identification of MART-1, Tyrosinase and SOX10 proteins 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:
MART-1 recognizes a protein of 18 kDa, identified at MART-1 (Melanoma Antigen Recognized by T cells 1). MART-1 is a useful addition to melanoma panels as it is specific for melanocytic lesions (1-3). Studies have shown that MART-1 is more sensitive than HMB45 when labeling metastatic melanomas (3). Tyrosinase is a key enzyme involved in the initial stages of melanin biosynthesis. Studies have shown Tyrosinase to be a more sensitive marker when compared to HMB45 and MART-1. It has also been shown to label a higher percentage of desmoplastic melanomas than HMB45 (1).The combination of MART-1 and Tyrosinase aids in identifying metastatic melanoma in sentinel lymph nodes (2).

The transcription factor SRY-related HMG-Box gene 10 (SOX10) plays an important role in neural crest, peripheral nervous system, and melanocytic cell development (4-8). SOX10 is widely expressed in normal human tissues including melanocytes and breast tissue. SOX10 is also an important marker in malignant tumors such as melanoma, breast carcinoma, gliomas, and benign tumors such as schwannomas (4-6). More importantly, SOX10 has been shown to be expressed in the vast majority of desmoplastic and spindle cell melanomas and has also been shown to be expressed in 100% of nevi (4,5). SOX10 is also less likely than S100 to be expressed by background fibroblasts and histiocytes within scars, and thus SOX10 may be superior to S100 in these types of cases (6).

The combination of SOX10 with MART-1 and/or Tyrosinase has been shown to stain a higher percentage of melanomas in lymph nodes and in metastatic melanoma compared to S100. It has also been shown to be more specific than S100, considering that S100 stains dendritic processes in lymph node while SOX10, MART-1 and Tyrosinase are negative (7,8). The cocktail of SOX10, MART-1 and Tyrosinase may be suitable for tumors of unknown origin or in differential diagnosis of melanoma and its mimics. PATENT PENDING.

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 secondary antibody is added to bind to the primary antibody. An enzyme label is then added to bind to the secondary antibody; this detection of the bound antibody is evidenced by a colorimetric reaction.

Reagent Provided:
MART-1 + Tyrosinase + SOX10 is provided as a prediluted antibody cocktail of anti- MART-1, anti-Tyrosinase and anti-SOX10 antibodies in buffer with carrier protein and preservative.

Antibody	anti-MART-1	anti-Tyrosinase	anti-SOX10
Clone	M2-7C10 + M2-9E3	T311	BC34
Source	Mouse monoclonal	Mouse monoclonal	Mouse monoclonal
Isotype	IgG2b	IgG2a	IgG1
Epitope/ Antigen	MART-1	Tyrosinase	SOX10
Cellular Localization	Cytoplasmic	Cytoplasmic	Nuclear
Staining	Brown (DAB)	Brown (DAB)	Brown (DAB)

Storage and Stability:
Store at 2ºC to 8ºC. 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 2ºC to 8ºC.

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Species Reactivity: Human; others not tested
Positive Tissue Control: Melanoma

Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 1.
Pretreatment: Perform heat retrieval using Reveal or Diva Decloaker. Refer to the Reveal or Diva Decloaker product data sheet for specific instructions.
Protein Block (Optional): Incubate for 5-10 minutes at RT with Background Punisher.
Primary Antibody: Incubate for 30 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 Notes:
This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

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

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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) (9)
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 in contact with sensitive areas, wash with copious amounts of water. (10)
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. Orchard G. Evaluation of melanocytic neoplasms: application of a pan-melanoma antibody cocktail. Br J Biomed Sci. 2002;59(4):196-202.
2. Cook MG, *et al.* The development of optimal pathological assessment of sentinel lymph nodes for melanoma. J Pathol. 2003 Jul;200(3):314-9.
3. Blessing K, Sanders DS, Grant JJ. Comparison of immunohistochemical staining of the novel antibody Melan-A with S100 protein and HMB-45 in malignant melanoma and melanoma variants. Histopathology. 1998 Feb; 32 (2):139-46.
4. Mohamed A, *et al.* SOX10 Expression in malignant melanoma, carcinoma, and normal tissues. Appl Immunohistochem Mol Morphol. 2013 Dec; 21(6):506-10.
5. Mollaaghababa R, Pavan WJ. The importance of having your SOX on: role of SOX10 in the development of neural crest-derived melanocytes and glia. Oncogene. 2003 May 19; 22(20):3024-34.
6. Tacha D, *et al.* A newly developed mouse monoclonal SOX10 antibody is a highly sensitive and specific marker for malignant melanoma, including spindle cell and desmoplastic melanomas. Arch Pathol Lab Med. 2015 Apr;139(4):530-6.
7. Tacha D, *et al.* An Immunohistochemical Comparison Study of SOX10, Pan Melanoma Cocktail and S100 in Malignant Melanoma. American Society of Dermatopathology, 51st Annual Meeting; Poster #278, Nov 6, 2014.
8. Willis BC, *et al.* SOX10: a useful marker for identifying metastatic melanoma in sentinel lymph nodes. Appl Immunohistochem Mol Morphol. 2015 Feb;23(2):109-12.
9. 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."
10. 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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