MART-1 + Tyrosinase + pHH3

Prediluted Multiplex Antibody Reagent
901-3186DS-090817

BIOCARE MEDICAL

Catalog Number:	**API 3186DS AA**
Description:	6.0 ml, prediluted
Dilution:	Ready-to-use
Diluent:	N/A

Intended Use:
For In Vitro Diagnostic Use
MART-1 + Tyrosinase + pHH3 is a cocktail of mouse and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of MART-1, Tyrosinase and phosphohistone H3 (Ser10) 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 apparently specific for melanocytic lesions (1,2). Studies have shown that MART-1 is more sensitive than HMB45 when labeling metastatic melanomas (3). This MART-1 cocktail does not stain steroid tumors like Melan A [103] does. Tyrosinase has been shown a more sensitive marker in recognizing melanoma 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 (4).
Microscopic evaluation of mitotic figures on H&E is a routine procedure in the assessment of the tumor grades (5). However, the counting of mitosis is manual and time consuming with assorted difficulties as well as variabilities between interobserver assessments (6). Histone H3 phosphorylation at Serine10 (pHH3) is in association with mitotic chromatin condensation in late G2 and M phase of the cell cycle. pHH3 can distinguish mitosis from apoptotic nuclei (7). The immunohistochemical staining of Serine10-pHH3 has been reported to be comparable to mitotic figures in the H&E section (8-11). The combination of monoclonal anti-pHH3 with MART-1 and Tyrosinase would offer an advantage of specific epitopes for melanoma diagnosis and mitosis counting.

Principle of Procedure:
This product is a primary antibody cocktail of mouse and rabbit antibodies, which may be used in a Multiplex IHC staining procedure to produce a two-color stain. Following application of the primary antibody cocktail to the tissue sample, detection is performed by separate secondary antibodies specific for each species (i.e. mouse or rabbit) of the primary antibody cocktail, which are conjugated to horseradish peroxidase (HRP) or alkaline phosphatase (AP) enzymes. Visualization is accomplished by the application of chromogenic substrates (DAB and Warp Red), which are enzymatically activated (by HRP or AP, respectively) to produce a colored reaction product at the antigen site. The specimen may be counterstained and coverslipped. Results are interpreted using a light microscope.

Reagent Provided:
MART-1 + Tyrosinase + pHH3 is provided as a prediluted antibody cocktail of anti-MART-1, anti-Tyrosinase and anti-pHH3 antibodies in buffer with carrier protein and preservative.

Antibody	anti-MART-1	anti-Tyrosinase	anti-pHH3
Clone	M2-7C10 + M2-9E3	T311	BC37
Source	Mouse monoclonal	Mouse monoclonal	Rabbit monoclonal
Isotype	IgG2b	IgG2a	IgG
Epitope/ Antigen	MART-1	Tyrosinase	PhosphoSer10 of Histone H3
Cellular Localization	Cytoplasmic	Cytoplasmic	Nuclear (mitotic figure)
Staining	Red (Warp Red)	Red (Warp Red)	Brown (DAB)

Storage and Stability:
Store at 2°C to 8°C. Do not use after expiration date printed on vial. If reagents are stored under conditions other than those specified in the package insert, they must be verified by the user. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)

Species Reactivity: Human; others not tested

Positive Tissue Control: Melanoma

Protocol Recommendations:
Peroxide Block:
Block for 5 minutes with Biocare's Peroxidazed 1.

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

Protein Block: Incubate for 10 minutes at RT with Biocare's Background Punisher.

Primary Antibody: Incubate for 30 minutes at RT.

Double Stain Detection: Incubate for 30 minutes at RT using Biocare's MACH 2 Double Stain 1.

Chromogen (1): Incubate for 5-7 minutes at RT with Biocare's Warp Red. Rinse in deionized water.

Chromogen (2): Incubate for 5 minutes at RT with Biocare's DAB.

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

Technical Note:
This antibody has been standardized with Biocare's MACH 2 Double Stain 1. Use TBS buffer for washing steps.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

EC REP EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

Tel: 800-799-9499 | www.biocare.net | Fax: 925-603-8080

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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. The clinical interpretation of any positive or negative staining should be evaluated within the context of clinical presentation, morphology and other histopathological criteria by a qualified pathologist. The clinical interpretation of any positive or negative staining should be complemented by morphological studies using proper positive and negative internal and external controls as well as other diagnostic tests.

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 (NaN_3) 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) (12)
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. (13)
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. Blessing K*, et al.* 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.
3. Cook MG, *et al.* The development of optimal pathological assessment of sentinel lymph nodes for melanoma. J Pathol. 2003 Jul;200(3):314-9.
4. 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.
5. Jannink I, *et al.* Comparison of the prognostic value of four methods to assess mitotic activity in 186 invasive breast cancer patients: classical and random mitotic activity assessments with correction for volume percentage of epithelium. Hum Pathol. 1995 Oct;26(10):1086-92.
6. Yadav KS, *et al.* Assessment of interobserver variability in mitotic figure counting in different histological grades of oral squamous cell carcinoma. J Contemp Dent Pract. 2012 May 1;13(3):339-44.
7. Ladstein RG, *et al.* Prognostic importance of the mitotic marker phosphohistone H3 in cutaneous nodular melanoma. J Invest Dermatol. 2012 Apr;132(4):1247-52.
8. Thareja S, *et al.* Analysis of tumor mitotic rate in thin metastatic melanomas compared with thin melanomas without metastasis using both the hematoxylin and eosin and anti-phosphohistone 3 IHC stain. Am J Dermatopathol. 2014 Jan;36(1):64-7.
9. Ikenberg K, *et al.* Immunohistochemical dual staining as an adjunct in assessment of mitotic activity in melanoma. J Cutan Pathol. 2012 Mar;39(3):324-30.
10. Casper DJ, *et al.* Use of anti-phosphohistone H3 immunohistochemistry to determine mitotic rate in thin melanoma. Am J Dermatopathol. 2010 Oct;32(7):650-4.
11. Fulton R, Tacha D. Use of a Novel Rabbit Monoclonal Phospho-Histone H3 (Ser10) versus H&E Mitotic Count in Melanoma. Mod Pathol 2016;29: (Suppl 2) 128A.
12. 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."
13. 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.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

IVD CE

EC REP EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands