Pan Melanoma + S100 (Tyrosinase + MART-1 + S100)

Prediluted Multiplex Cocktail (4-Step)
9012-213DS-071717

BIOCARE MEDICAL

Catalog Number:	**APR 213DS AA, H**
Description:	6.0, 25 ml, prediluted
Dilution:	Ready-to-use
Diluent:	N/A

Intended Use:
For Research Use Only. Not for use in diagnostic procedures.

Summary and Explanation:
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 shown to label a higher percentage of desmoplastic melanomas than HMB45.

MART-1 Cocktail (M2-7C10 + M2-9E3) recognizes a protein of 18kDa, identified as 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. Studies have also shown that MART-1 is more sensitive than HMB45 when labeling metastatic melanomas. This MART-1 cocktail does not stain steroid tumors unlike Melan A [103].

S100 stains Schwannomas, ependymomas, astrogliomas, almost all benign and malignant melanomas and their metastases. S100 protein is also expressed in the antigen presenting cells such as the Langerhans cells in skin and interdigitating reticulum cells in the paracortex of lymph nodes.

Principle of Multiplex Staining:
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 universal, affinity-purified, 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.

Source: Mouse monoclonal and Rabbit polyclonal
Species Reactivity: Human; others not tested.
Clone: T311 + M2-7C10 + M2-9E3+N/A
Isotype: IgG_{2a}+ $IgG_{2b/kappa}$ + $IgG_{2b\ /kappa}$ + N/A
Epitope/Antigen: Tyrosinase, MART-1, S100 protein
Cellular Localization:
Tyrosinase: (cytoplasmic); brown, MART-1: (cytoplasmic); brown
S100: (nuclear/cytoplasmic); red
Positive Control: Melanoma
Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Supplied As: Buffer with protein carrier and preservative.
Storage and Stability:
Store at 2ºC to 8ºC. 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 2ºC to 8ºC.

Staining Protocol Recommendations:
Peroxide Block:
Block for 5 minutes with Biocare's Peroxidazed 1.
Pretreatment Solution (recommended): Diva or Reveal
Pretreatment Protocol:
Heat Retrieval Method:
Retrieve sections under pressure using Biocare's Decloaking Chamber, followed by a wash in distilled water, alternatively, steam tissue for 45-60 minutes. Allow solution to cool for 10 minutes then wash in distilled water.
Protein Block (Optional): Incubate for 10-15 minutes at RT with Biocare's Background Sniper.
Primary Antibody:
Incubate for 30-60 minutes at RT.
Double Stain Detection:
Incubate for 30 minutes at RT using Biocare's MACH 2 Double Stain 2.
Chromogen (1): Incubate for 5 minutes at RT when using BIOCARE's Betazoid DAB.
Chromogen (2):
Incubate for 10-20 minutes at RT with BIOCARE's Vulcan Fast Red.
Counterstain:
Rinse with deionized water. Incubate for 30-60 seconds with Hematoxylin. Rinse with deionized water. Apply Tacha's Bluing solution for 1 minute.
Technical Notes:
This antibody has been standardized with BIOCARE's MACH 2 Double Stain 2. It can also be used on an automated staining system. Use TBS buffer for washing steps.
Precautions:
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)
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 membrane with reagents and specimens. If reagents or specimens come in contact with sensitive areas, wash with copious amounts of water. Microbial contamination of reagents may result in an increase in nonspecific staining Incubation times or temperatures other than those specified may give erroneous results. The user must validate any such change. The MSDS is available upon request.
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. Shidham VB et al., Improved immunohistochemical evaluation of micrometastases in sentinel lymph nodes of cutaneous melanoma with 'MCW melanoma cocktail'—a mixture of monoclonal antibodies to MART-1, Melan-A, and tyrosinase. BMC Clin Pathol. 2007 Mar 7;7:2.
2. Orchard G. Evaluation of melanocytic neoplasms: application of a pan-melanoma antibody. Br J Biomed Sci. 2002;59(4):196-202
3. 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."

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

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

References Cont'd:

4. National Committee for Clinical Laboratory Standards(NCCLS). Protection of laboratory workers from infectious diseases transmitted by blood and tissue; proposed guideline. Villanova, PA 1991;7(9). Order code M29-P.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

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