Pan Melanoma + Ki-67
Prediluted Multiplex Cocktail (4-Step)

Catalog Number: PM 362 DS AA, H
Description: 6.0, 25ml, prediluted
Dilution: Ready-to-use
Diluent: N/A

Intended Use:
For In Vitro Diagnostic Use

Summary and Explanation:
This Multiplex application serves as a tool to identify the proliferation rate of melanocytic lesions in cases in which melanocytes are sparse; there are dense lymphocytic infiltrates; and melanocytes are admixed with fibroblasts. In general, a higher proliferative fraction is seen in melanoma than in melanocytic nevi. There are many types of nevi, and some simulate melanoma closely. If the Multiplex stain shows a very low Ki-67 (DAB) labeling rate in MART-1/Tyrosinase positive cells (Fast Red), this favors benignity. A high rate, especially toward the deep part of a melanocytic lesion raises the possibility of malignancy.

Principle of Multiplex Staining:
A Multiplex IHC stain can be accomplished in four major steps. The initial step consists of an antibody cocktail with at least one mouse and one rabbit antibody. This cocktail is applied to the tissue and will bind with two or more target antigens. A multiplex detection cocktail of horseradish peroxidase (HRP) and alkaline phosphatase (AP) conjugated secondary antibodies is applied. The third step consists of the addition of DAB-Substrate that binds to the HRP and produces a brown chromogenic reaction product. The fourth step consists of a Fast Red-Substrate that binds to the AP and produces a red chromogenic reaction product.

Source: Mouse monoclonal and Rabbit monoclonal
Species Reactivity: Human; others not tested
Clone: M2-7C10 + M2-9E3 + T3-11 + SP6
Isotype: IgG2a + IgG2b/kappa + IgG2b/kappa + rabbit IgG
Epitope/Antigen: Melanoma / Ki-67

Cellular Localization:
MART-1 + Tyrosinase: (Cytoplasmic) Red
Ki-67: (Nuclear) Brown

Positive Control: Melanoma

Known Applications:
Immunohistochemistry (formalin-fixed paraffin-embedded tissues)
Supplied As: Buffer with protein carrier and preservative.

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.

Protocol Recommendations
Peroxide Block:
Block for 5 minutes with Biocare's Peroxidazed 1.
Normal Tissue: Nevus
Abnormal Tissue: Melanoma

Pretreatment Solution (recommended): Diva

Pretreatment Protocol:
Heat Retrieval Method:
Preheat the retrieval solution to 95°C for 30 minutes in Biocare's Decloaking Chamber. Then, place slides into the preheated solution and retrieve under pressure at 95°C for 40 minutes. Alternatively, steam tissue sections for 45-60 minutes or use a water bath at 95°C for 40 minutes. Allow solution to cool for 20 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 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 minutes at RT when using Biocare's Betazoid DAB.

Chromogen (2): Incubate for 10-20 minutes at RT with Biocare's Vulcan Fast Red. Rinse in deionized water.

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:
1. This antibody has been standardized with Biocare's MACH 2 Double Stain 1 detection system. It can also be used on an automated staining system. Use TBS buffer for washing steps. 2. Fix tissues 12-24 hours. Shorter fixation times may cause tissue to fall off the slide or cause poor morphology. 3. We do not recommend antigen retrieval temperatures above 95°C.

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. These products are tools that can be used for interpretation of morphological findings in conjunction with other diagnostic tests and pertinent clinical data by a qualified pathologist.

Quality Control:
Refer to NCCLS Quality Assurance for Immunocytochemistry approved guidelines, December 1999 MM44-A Vol.19 No.26 for more information about tissue controls.

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 membranes 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.
References:

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.