

**Pan Melanoma + Ki-67**  
Prediluted Multiplex Cocktail (4-Step)  
Control Number: 902-362DS-090517

**Catalog Number:** APR 362 DS 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:**

This Multiplex IHC application may serve 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. According to studies, a higher proliferative fraction is seen in melanoma than in melanocytic nevi. There are many types of nevi, and some simulate melanoma closely. Studies have shown that 1) If the Multiplex stain shows a very low Ki-67 (DAB) labeling rate in MART-1/Tyrosinase positive cells (Fast Red), this favors benignity. 2) A high rate, especially toward the deep part of a melanocytic lesion raises the possibility of malignancy.

**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.

**Staining Protocol Recommendations:**

**Peroxide Block:** Block for 5 minutes with Biocare's Peroxidized 1.

**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 Note:**

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:**

This product is provided for Research Use Only (RUO) and is not for use in diagnostic procedures. Suitability for specific applications may vary and it is the responsibility of the end user to determine the appropriate application for its use.

**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<sub>3</sub>) 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)

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.

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 MSDS is available upon request and is located at <http://biocare.net/support/msds/>.

**Technical Support:**

Contact Biocare's Technical Support at 1-800-542-2002 for questions regarding this product.

**References:**

1. Orchard G. Evaluation of melanocytic neoplasms: application of a pan-melanoma antibody cocktail. *Br J Biomed Sci.* 2002; 59(4):196-20.

2. Orchard GE. Melan A (MART-1): a new monoclonal antibody for malignant melanoma diagnosis. *Br J Biomed Sci* 1998 Mar; 55(1):9-9.

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):139-146.

4. Jansen R, *et al.* MIB-1 labelling index is an independent prognostic marker in primary breast cancer. *Br J Cancer* 1998 Aug; 78 (4):460-465.

5. Goodson WH 3rd, *et al.* The functional relationship between in vivo bromodeoxyuridine labeling index and Ki-67 proliferation index in human breast cancer. *Breast Cancer Res Treat* 1998 May; 49 (2): 155-164.

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-Third Edition CLSI document M29-A3 Wayne, PA 2005