

# Pan Melanoma + Ki-67

Prediluted Multiplex Antibody Reagent  
901-362DS-060223

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| Available Product Formats |                 |             |              |         |
|---------------------------|-----------------|-------------|--------------|---------|
| Format                    | Catalog Number  | Description | Dilution     | Diluent |
| Predilute                 | PM 362 DS AA, H | 6.0, 25 mL  | Ready-to-use | N/A     |
| ONCORE Pro                | OPAI 362 T60    | 60 tests    | Ready-to-use | N/A     |
| VALENT                    | VTMR 362 G20    | 20 mL       | Ready-to-use | N/A     |

## Intended Use:

For In Vitro Diagnostic Use

Pan Melanoma + Ki-67 is a cocktail of mouse monoclonal and rabbit monoclonal antibodies that is intended for laboratory use in the qualitative identification of MART-1, Tyrosinase and Ki-67 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:

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 (Red), this favors benignity. A high rate, especially toward the deep part of a melanocytic lesion raises the possibility of malignancy.

## 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 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 cover slipped. Results are interpreted using a light microscope.

## Reagent Provided:

Pan Melanoma + Ki-67 is provided as a prediluted antibody cocktail of anti-MART-1, anti-Tyrosinase and anti-Ki-67 antibodies, in buffer with carrier protein and preservative.

| Antibody                 | anti-MART-1      | anti-Tyrosinase  | anti-Ki-67        |
|--------------------------|------------------|------------------|-------------------|
| Clone                    | M2-7C10 + M2-9E3 | T311             | SP6               |
| Source                   | Mouse Monoclonal | Mouse Monoclonal | Rabbit Monoclonal |
| Isotype                  | IgG2b + IgG2b    | IgG2a            | IgG               |
| Epitope/<br>Antigen      | MART-1           | Tyrosinase       | Ki-67             |
| Cellular<br>Localization | Cytoplasmic      | Cytoplasmic      | Nuclear           |
| Staining                 | Red              | Red              | Brown (DAB)       |

## Storage and Stability:

Store at 2°C to 8°C. The product is stable to the expiration date printed on the label, when stored under these conditions.

## Known Applications:

immunohistochemistry (formalin-fixed paraffin-embedded tissues)

**Species Reactivity:** Human; others not tested

**Positive Tissue Control:** Melanoma

## Protocol Recommendations (VALENT® Automated Slide Staining Platform):

VTMR362 is intended for use with the VALENT. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Manager should be programmed as follows:

**Deparaffinization:** Deparaffinize for 8 minutes with Val DePar.

**Pretreatment:** Perform heat retrieval at 98°C for 60 minutes using Val AR-Lo pH, 5X (use at 1X).

**Peroxidase Block:** Block for 5 minutes with Val Peroxidase Block.

**Protein Block:** Incubate for 10 minutes with Val Background Block.

**Primary Antibody:** Incubate for 45 minutes.

**Double Stain Detection:** Incubate for 30 minutes using Val Plex 1.

**Chromogen (1):** Incubate for 5 minutes with Val DAB.

**Chromogen (2):** Incubate for 15 minutes with Val Fast Red.

**Counterstain:** Counterstain for 5 minutes with Val Hematoxylin.

## Protocol Recommendations (intelliPATH FLX® and manual use):

**Peroxidase Block:** Block for 5 minutes with Peroxidase 1.

**Pretreatment:** Perform heat retrieval using Diva Decloaker. Refer to the Diva Decloaker product data sheet for specific instructions.

**Protein Block:** Incubate for 10 minutes at RT with Background Punisher.

**Primary Antibody:** Incubate for 30 minutes at RT.

**Double Stain Detection:** Incubate for 30 minutes at RT using MACH 2 Double Stain 1.

**Chromogen (1):** Incubate for 5 minutes at RT with Betazoid DAB.

**Chromogen (2):** Incubate for 5-7 minutes at RT with Warp Red. Rinse in deionized water.

## Counterstain:

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

## Technical Notes:

1. This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 2 Double Stain 1. Use TBS 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.

## Protocol Recommendations (ONCORE™ Pro Automated Slide Staining System):

OPAI362 is intended for use with the ONCORE Pro. Refer to the User Manual for specific instructions for use. Protocol parameters in the Protocol Editor should be programmed as follows:

**Protocol Name:** Pan Mel/Ki67

**Protocol Template (Description):** Multiplex 1 Template 1

**Dewaxing (DS Buffer Option):** DS2-50

**Antigen Retrieval (AR Option):** AR2, low pH; 101°C

**Block Option:** Buffer

**Reagent Name, Time, Temp.:** Pan Mel/Ki67, 30 min., 25°C

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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.

## 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](http://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<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) (6)
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. (7)
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):196-20.
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-Fourth Edition CLSI document M29-A4 Wayne, PA 2014.