

# Melan A (M)

Concentrated and Prediluted Monoclonal Antibody  
901-3114-052623

**BIOCARE**  
M E D I C A L

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACI 3114 A, B	0.1, 0.5 mL	1:25	Van Gogh Yellow
Predilute	API 3114 AA, H	6.0, 25 mL	Ready-to-use	N/A
ONCORE Pro	OPAI 3114 T60	60 tests	Ready-to-use	N/A
VALENT	VLTM 3114 G20	20 mL	Ready-to-use	N/A
UltraLine – For BenchMark	AVI 3114 G, G25	6.0, 25 mL	Ready-to-use	N/A
Q Series– For Leica BOND-III	ALI 3114 G7	7.0 mL	Ready-to-use	N/A

## Intended Use:

For In Vitro Diagnostic Use

Melan A (M) [A103] is a mouse monoclonal antibody that is intended for laboratory use in the qualitative identification of Melan A protein 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:

Melan-A (MART-1) [A103], a melanoma-specific antigen, is a transmembrane protein and a melanocyte differentiation marker recognized by cytotoxic T lymphocytes. Melan-A is expressed in skin, in the majority of melanocytes and in renal angiomyolipomas (1-3). The Melan-A A103 clone, unlike clones M2-7C10 and M2-9E3, can also aid in the recognition of steroid hormone-producing tumors and may be particularly useful in the diagnosis of adrenocortical carcinoma (4,5).

## Principle of Procedure:

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 one-, two- or three-step detection procedure can be employed. The one-step procedure will feature an enzyme-labeled polymer that binds to the primary antibody. A two-step procedure will feature a secondary antibody added to bind to the primary antibody. An enzyme-labeled polymer is then added to bind to the secondary antibody. The three-step detection procedure will feature a secondary antibody added to bind to the primary antibody followed by a linker antibody step for maximum binding. An enzyme-labeled polymer is then added to bind to the linker antibody. These detections of the bound antibodies are evidenced by a colorimetric reaction.

**Source:** Mouse monoclonal

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

**Clone:** A103

**Isotype:** IgG1

**Protein Concentration:** Call for lot specific Ig concentration.

**Epitope/Antigen:** Melan A

**Cellular Localization:** Cytoplasmic

**Positive Tissue 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. The product is stable to the expiration date printed on the label, when stored under these conditions. Do not use after expiration date. Diluted reagents should be used promptly; any remaining reagent should be stored at 2°C to 8°C.

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

VLTM3114 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:

### - DAB Chromogen Staining Option:

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

## Protocol Recommendations (VALENT Automated Slide Staining Platform) Cont'd:

**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 (Optional):** Incubate for 10-20 minutes at RT with Val Background Block.

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

**Secondary:** Incubate for 10 minutes with Val Mouse Secondary.

**Linker:** Incubate for 10 minutes with Val Universal Linker.

**Polymer:** Incubate for 10 minutes with Val Universal Polymer.

**Chromogen:** Incubate for 5 minutes with Val DAB.

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

### - Red Chromogen Staining Option:

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

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

**Protein Block (Optional):** Incubate for 10-20 minutes with Val Background Block.

**Primary Antibody:** Incubate for 60 minutes.

**Polymer:** Incubate for 45 min with Val Mouse AP Polymer.

**Chromogen:** Incubate for 15 min with Val Fast Red.

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

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

**Peroxide Block:** Block for 5 minutes with Peroxidized 1.

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

**Protein Block (Optional):** Incubate for 5-10 minutes at RT with Background Punisher.

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

**Probe:** Incubate for 10 minutes at RT with a secondary probe.

**Polymer:** Incubate for 10-20 minutes at RT with a tertiary polymer.

**Chromogen:** Incubate for 5 minutes at RT with Biocare's DAB – OR – Incubate for 5-7 minutes at RT with Warp Red.

### Counterstain:

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

### Technical Note:

This antibody, for intelliPATH FLX and manual use, has been standardized with MACH 4 detection system. Use TBS for washing steps.

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

OPAI3114 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:** Melan A

**Protocol Template (Description):** Ms HRP Template 1 – OR – Ms AP Template 1

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

**Antigen Retrieval (AR Option):** AR1, high pH; 103°C

**Block Option:** Buffer

**Reagent Name, Time, Temp.:** Melan A, 30 min., 25°C

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## **Protocol Recommendations (Ventana BenchMark ULTRA):**

AVI3114 is intended for use with the BenchMark ULTRA. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

- Using **OptiView**:

**Template/Detection:** OptiView DAB IHC

**Pretreatment Protocol:** CC1 64 minutes

**Peroxidase:** Pre Primary Peroxidase Inhibitor

**Primary Antibody:** 32 minutes, 36°C

- Using **ultraView AP Red**:

**Template/Detection:** ultraView Red

**Pretreatment Protocol:** CC1 Standard

**Primary Antibody:** 32 minutes, 37°C

## **Protocol Recommendations (Q Series – For Leica BOND-III):**

ALI3114 is intended for use with the Leica BOND-III. Refer to the User Manual for specific instructions for use. Recommended protocol parameters are as follows:

- **DAB Chromogen Staining Option:**

**Protocol Name:** IHC Protocol F

**Detection:** Bond Polymer Refine

**HIER:** 10 min with ER1

**Peroxide Block:** 5 min

**Marker (Primary Antibody):** 15 min

**Post Primary:** 8 min

**Polymer:** 8 min

**Mixed DAB Refine:** 10 min

**Hematoxylin:** 5 min

- **Red Chromogen Staining Option:**

**Protocol Name:** IHC Protocol J

**Detection:** Bond Polymer Refine Red

**HIER:** 10 min with ER1

**Marker (Primary Antibody):** 15 min

**Post Primary AP:** 20 min

**Polymer AP:** 30 min

**Mixed Red Refine:** 10 min + 5 min

**Hematoxylin:** 5 min

## **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). 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 into contact with sensitive areas, wash with copious amounts of water. (7)

## **Precautions Cont'd:**

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. Shidham VB, *et al.* Evaluation of micrometastases in sentinel lymph nodes of cutaneous melanoma: higher diagnostic accuracy with Melan-A and MART-1 compared with S-100 protein and HMB-45. *Am J Surg Pathol.* 2001 Aug;25(8):1039-46.

2. Zubovits J, *et al.* HMB-45, S-100, NK1/C3, and MART-1 in metastatic melanoma. *Hum Pathol.* 2004 Feb; 35(2):217-23.

3. Tuna EB, Lebe B, Yörükoğlu K. HMB45 and melan-A expression in renal angiomyolipoma and their significance for the diagnosis. *Tumori.* 2003 Jan-Feb; 89 (1):46-8.

4. Busam KJ, *et al.* Immunoreactivity for A103, an antibody to melan-A (Mart-1), in adrenocortical and other steroid tumors. *Am J Surg Pathol.* 1998 Jan; 22(1):57-63.

5. Zhang HY, *et al.* [Diagnostic value of A103 and inhibin-alpha in adrenocortical tumors: an immunohistochemical study using tissue microarray techniques]. *Zhonghua Bing Li Xue Za Zhi.* 2004 Jun; 33(3):203-7.

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.

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