Melan A (M)

Concentrated and Prediluted Monoclonal Antibody
902-3114-081021

BIOCARE MEDICAL

Available Product Formats				
Format	Catalog Number	Description	Dilution	Diluent
Concentrate	ACR 3114 A, B	0.1, 0.5 mL	1:25	Van Gogh Yellow
Predilute	APR 3114 AA, H	6.0, 25 mL	Ready-to-use	N/A
UltraLine – For BenchMark	AVR 3114 G, G25	6.0, 25 mL	Ready-to-use	N/A
Q Series– For Leica BOND-III	ALR 3114 G7	7.0 mL	Ready-to-use	N/A

Intended Use:
For Research Use Only. Not for use in diagnostic procedures.
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 2ºC to 8ºC. 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 2ºC to 8ºC.

Staining Protocol Recommendations (intelliPATH FLX® and manual use):
Peroxide Block: Block for 5 minutes with Peroxidazed 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.

Staining Protocol Recommendations (intelliPATH FLX and manual use) Cont'd:
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.

Staining Protocol Recommendations (Ventana BenchMark ULTRA):
AVR3114 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 ***ultra*View AP Red:**

Template/Detection: ultraView Red
Pretreatment Protocol: CC1 Standard
Primary Antibody: 32 minutes, 37°C

Staining Protocol Recommendations (Q Series – For Leica BOND-III):
ALR3114 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

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

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

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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_3) 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)
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.

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

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

References Cont'd:
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.

Biocare Medical
60 Berry Drive
Pacheco, CA 94553
USA

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