Novocastra™ Ready-to-Use Mouse Monoclonal Antibody Melan A

Product Code: RTU-MelanA

Intended Use	FOR RESEARCH USE ONLY.
Specificity	Human melan A, recognising a 20-22 kD doublet in melan A mRNA-positive melanoma cell lines. Does not react with melan A mRNA-negative cell lines. (Chen et al., 1996).
Clone	A103
Ig Class	IgG1
Antigen Used for Immunizations	Prokaryotic recombinant protein corresponding to melan A molecule.
Hybridoma Partner	Mouse myeloma (Sp2/0-Ag14).
Preparation	Tissue culture supernatant diluted in 5% horse serum in PBS containing 12 mM sodium azide. Volume as indicated on vial label.
Effective on Frozen Tissue	Yes. Acetone fixation recommended.
Effective on Paraffin Wax Embedded Tissue	Yes (using the high temperature antigen unmasking technique: see overleaf).
Recommendations on Use	Immunohistochemistry: Typical working dilution: neat. 15 minutes primary antibody incubation at 25 °C when used in conjunction with the Novostain Universal Detection Kit (Ready to Use), code NCL-RTU-D. Recommendations on use will differ if other detection systems are used eg Standard ABC technique. Western Blotting: Not recommended. Not recommended for use on Ventana automated staining systems (Ventana Medical Systems Inc., USA).
Positive Controls	Immunohistochemistry: Skin melanocytes
Staining Pattern	Cytoplasmic
Storage and Stability	Store ready-to-use prediluted liquid antibody at 4 °C. Return to 4 °C immediately after use. Under these conditions, there is no significant loss in product performance up to the expiry date indicated on the vial label.
General Overview	Melan A, a product of the MART-1 gene, is a melanocyte differentiation antigen recognised by autologous cytotoxic T lymphocytes. It is a relatively small protein of 118 amino acids and its expression is limited to cells of melanocyte lineage and retinal pigment epithelium. Melan A is thought to be a transmembrane protein and cell fractionation studies have suggested that it is present in melanosomes and endoplasmic reticulum.
General References	Clarkson K S, Sturdgess I C and Molyneux A J. Journal of Clinical Pathology. 54: 196–200 (2001). de Vries T J, Smeets M, de Graaf R, et al.. Journal of Pathology. 193: 13–20 (2001). Chen Y-T, Stockert E, Jungbluth A, et al.. Proceedings of the National Academy of Sciences USA. 93: 5915–5919 (1996). Jäger E, Ringhoffer M, Karback J, et al.. International Journal of Cancer. 66: 470–476 (1996).

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Instructions for Use

High Temperature Antigen Unmasking Technique for Immunohistochemical Demonstration on Paraffin Sections

1. Cut and mount sections on slides coated with a suitable tissue adhesive.
2. Deparaffinize sections and rehydrate to distilled water.
3. Place sections in 0.5% hydrogen peroxide/methanol for 10 minutes (or use other appropriate endogenous peroxidase blocking procedure). Wash sections in tap water.
4. Heat 1500 mL of the recommended unmasking solution (0.01 M citrate buffer, pH 6.0 (or Epitope Retrieval Solution, RE7113) unless otherwise indicated overleaf) until boiling in a stainless steel pressure cooker. Cover but do not lock lid.
5. Position slides into metal staining racks (do not place slides close together as uneven staining may occur) and lower into pressure cooker ensuring slides are completely immersed in unmasking solution. Lock lid.
6. When the pressure cooker reaches operating temperature and pressure (after about 5 minutes) start a timer for 1 minute (unless otherwise indicated on the data sheet).
7. When the timer rings, remove pressure cooker from heat source and run under cold water with lid on. DO NOT OPEN LID UNTIL THE INDICATORS SHOW THAT PRESSURE HAS BEEN RELEASED. Open lid, remove slides and place immediately into a bath of tap water.
8. Wash sections in TBS* buffer (pH 7.6) for 1 x 5 minutes.
9. Place sections in diluted normal serum (or RTU Normal Horse Serum) for 10 minutes.
10. Incubate sections with primary antibody. Use Antibody Diluent RE7133 (where available).
11. Wash in TBS buffer for 2 x 5 minutes.
12. Incubate sections in an appropriate biotinylated secondary antibody.
13. Wash in TBS buffer for 2 x 5 minutes.
14. Incubate slides in ABC reagent (or RTU streptavidin/peroxidase complex).
15. Wash in TBS buffer for 2 x 5 minutes.
16. Incubate slides in DAB or other suitable peroxidase substrate.
17. Wash thoroughly in running tap water.
18. Counterstain with hematoxylin (if required), dehydrate and mount.

Solutions

0.01 M CITRATE BUFFER (pH 6.0) or RE7113 (where available).
Add 3.84 g of citric acid (anhydrous) to 1.8 L of distilled water. Adjust to pH 6.0 using concentrated NaOH. Make up to 2 L with distilled water.

1 mM EDTA (pH 8.0) or RE7116 (where available).
Add 0.37 g of EDTA (SIGMA product code E-5134) to 1 litre of distilled water. Adjust pH to 8.0 using 1.0 M NaOH.

20 mM TRIS/ 0.65 mM EDTA/ 0.005% TWEEN (pH 9.0) or RE7119 (where available).
Dissolve 14.4 g Tris (BDH product code 271197K) and 1.44 g EDTA (SIGMA product code E-5134) to 0.55 L of distilled water. Adjust pH to 9.0 with 1 M HCl and add 0.3 mL Tween 20 (SIGMA product code P-1379). Make up to 0.6 L with distilled water. This is a 10x concentrate which should be diluted with distilled water as required (eg 150 mL diluted with 1350 mL of distilled water).

** In most applications, 10 mM phosphate, 0.15 M NaCl, pH 7.6 (PBS) can be used instead of 50 mM Tris, 0.15 M NaCl, pH 7.6 (TBS).*

Safety Note

To ensure the correct and safe use of your pressure cooker, PLEASE READ MANUFACTURER'S INSTRUCTIONS.