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Part 3580 Revised January 2020

Melan-A (MART-1) Control Slides – Technical Memo

Part 3580B 98 Slide/Set

PRODUCT SPECIFICATIONS:

Tissue: Positive staining melanoma and negative staining lung.

Fixation: Formalin 10%, Phosphate Buffered (Part 1090).

Section/Glass: Paraffin sections cut at 4 microns on Superfrost™ Plus slides.

Quality Control Stain: Melan-A (MART-1) quality control stained slide(s) included.

Reactivity: Guaranteed product specific reactivity for one year from date of receipt. Revalidate after one year to verify continued reactivity.

Storage: 15-30°C in a light deprived and humidity controlled environment.

Part 3580A 10 Slide/Set

Intended Use: To verify histological techniques and reagent reactivity.

Before using unstained control slides, review the enclosed stained slide(s) to ensure that this tissue source is acceptable for testing needs.

APPLICATION:

Newcomer Supply Melan-A (MART-1) Control Slides are for the positive immunohistochemical staining of Melan-A, expressed in melanocytes of normal skin, retina, nevi, and in the vast majority of melanomas, and is useful in establishing the diagnosis of metastatic melanomas.

NEWCOMER SUPPLY VALIDATION PROCEDURE:

- 1. Heat dry sections in oven according to your laboratory protocol.
- Deparaffinize sections thoroughly in three changes of xylene, 3 2. minutes each. Hydrate through two changes each of 100% and 95% ethyl alcohols, 10 dips each. Wash well with distilled water. See Procedure Note #1. а.
- Proceed, if necessary, with an epitope/antigen retrieval technique 3. approved for use in your laboratory.
- Rinse in distilled water; tap off excess water. 4
- 5. Circle sections with Pap Pen Liquid Blocker (Part 6505, 6506 or 6507) to reduce reagent usage and ensure tissue coverage.
- 6. Block endogenous peroxidase with freshly made 3% Hydrogen Peroxide. Incubate for 5 minutes.
 - See Procedure Note #2. a.
- Wash slides gently in distilled water. Rinse in two changes of Tris 7. Buffered Saline.
 - a. See Procedure Note #3.
- Tap off excess buffer; apply MART-1 primary antibody. Incubate 8. at room temperature for 30 minutes. 9
 - Rinse slides in two changes of buffer.
- Tap off excess buffer; apply Amplifier. Incubate for 10 minutes. 10.
- Rinse slides in two changes of buffer. 11.
- Tap off excess buffer; apply HRP Polymer. Incubate for 10 12. minutes.
- Rinse slides in two changes of buffer. 13.
- Prepare required quantity of DAB substrate/chromogen. 14.
- 15. Tap off excess buffer; apply DAB. Incubate for 5 minutes.
- Rinse slides in four changes of distilled water. 16.
- Counterstain lightly with Hematoxylin Stain, Gill I (Part 1180) for 5 17. minutes.
- 18. Rinse slides in warm tap water to blue sections.
- Dehydrate in two changes each of 95% and 100% ethyl alcohol. 19. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

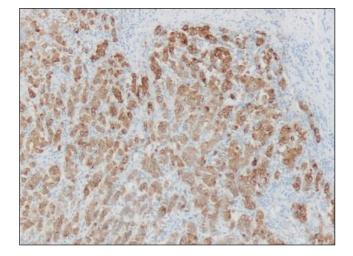
Melan-A positive expression	Brown cytoplasmic staining
Lung	Negative
Nuclei	Blue

PROCEDURE NOTES:

- Do not allow sections to dry out at any point during procedure.
- Dilute sufficient Hydrogen Peroxide 30%, Aqueous (Part 1206) with 2. distilled water to a 3% (1/10) solution prior to use.
- 3. Dilute sufficient Tris Buffered Saline 0.05M, pH 7.6, 10X (Part 140304) with distilled water to a 1/10 solution prior to use for all buffer rinses in this procedure.
- Cell Marque MART-1 (A103) is the concentrated primary antibody 4 used. Dilute primary antibody to a 1/50 working dilution with Cell Marque Emerald: Antibody Diluent (936B).
- 5. Cell Margue HiDef Detection[™] HRP Polymer System (954D) provides the Amplifier and HRP Polymer solutions used.
- Cell Marque DAB Substrate Kit (957D) is the chromogen used. 6.
- If using a xylene substitute, closely follow the manufacturer's 7 recommendations for deparaffinization and clearing steps.

REFERENCES:

- Cell Marque MART-1 Antibody datasheet. 1.
- 2. Cell Marque Emerald: Antibody Diluent datasheet.
- 3. Cell Marque HiDef Detection[™] Polymer System datasheet.
- Cell Marque DAB Substrate Kit datasheet. 4
- 5. Modifications developed by Newcomer Supply Laboratory.



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