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Part 3185 Revised January 2025

CD68 Control Slides – Technical Memo

CONTROL SLIDES: Part 3185A Part 3185B 10 Slide/Set 98 Slide/Set

PRODUCT SPECIFICATIONS:

Tissue: Positive staining tonsil and negative staining myometrium.

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

Section/Glass: Paraffin sections cut at 4 microns on Superfrost™ Plus slides. Quality Control Stain: CD68 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. **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 CD68 Control Slides are for the positive immunohistochemical staining of CD68, a useful marker for cells of macrophage lineage, including monocytes and histiocytes. Kupffer cells and osteoclasts are also stained.

NEWCOMER SUPPLY VALIDATION PROCEDURE:

- 1. Heat dry sections in oven according to your laboratory protocol.
- Deparaffinize sections thoroughly in three changes of xylene, 3
 minutes each. Hydrate through two changes each of 100% and
 95% ethyl alcohols, 10 dips each. Wash well with distilled water.
 - a. See Procedure Note #1.
- 3. Proceed with an epitope/antigen retrieval technique approved for use in your laboratory.
- 4. Rinse in distilled water; tap off excess water.
- Circle sections with Pap Pen Liquid Blocker (Part 6505, 6506 or 6507) to reduce reagent usage and ensure tissue coverage.
- Block endogenous peroxidase with freshly made 3% Hydrogen Peroxide. Incubate for 5 minutes.
 - a. See Procedure Note #2.
- Wash slides gently in distilled water. Rinse in two changes of Tris Buffered Saline.
 - a. See Procedure Note #3.
- 8. Tap off excess buffer; apply CD68 primary antibody. Incubate at room temperature for 30 minutes.
- 9. Rinse slides in two changes of buffer.
- 10. Tap off excess buffer; apply Amplifier. Incubate for 10 minutes.
- 11. Rinse slides in two changes of buffer.
- 12. Tap off excess buffer; apply HRP Polymer. Incubate for 10 minutes.
- 13. Rinse slides in two changes of buffer.
- 14. Prepare required quantity of DAB substrate/chromogen.
- 15. Tap off excess buffer; apply DAB. Incubate for 5 minutes.
- 16. Rinse slides in four changes of distilled water.
- 17. Counterstain with Hematoxylin Stain, Gill I (Part 1180); 1-10 dips.
- 18. Rinse slides in warm tap water to blue sections.
- Dehydrate in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.

RESULTS:

CD68 positive expression Brown cytoplasmic & membrane staining Myometrium Negative

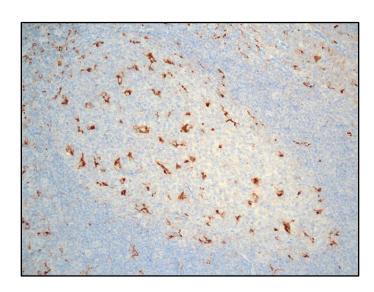
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PROCEDURE NOTES:

- Do not allow sections to dry out at any point during procedure.
- Dilute Hydrogen Peroxide 30% Aqueous Solution (Part 1206) with distilled water to a 3% (1/10) solution prior to use.
- 3. Dilute 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.
- 4. Cell Marque CD68 (Kp-1) is the primary antibody used along with Cell Marque detection and ancillary reagents.
- 5. If using a xylene substitute, follow manufacturer's recommendation for deparaffinization and clearing steps.

REFERENCES:

- Cell Marque CD68 Antibody datasheet.
- 2. Modifications developed by Newcomer Supply Laboratory.



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