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Part 3746
Revised January 2020

Spirochete *Treponema sp.*, Artificial Control Slides – Technical Memo

CONTROL SLIDES:

Part 3746A	**Part 3746B**
10 Slide/Set	98 Slide/Set

PRODUCT SPECIFICATIONS:
Tissue: Positive staining rat lung and negative staining human lung.
Fixation: Formalin 10%, Phosphate Buffered (Part 1090).
Section/Glass: Paraffin sections cut at 4 microns on Superfrost™ Plus slides.
Quality Control Stain: Spirochete 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 Spirochete *Treponema sp.,* Artificial Control Slides are for the positive immunohistochemical staining of spirochetes, the causative agent of a variety of diseases such as; syphilis, bejel, pinta, yaws and lyme. *Treponema hyodysenteriae* purchased from American Type Culture Collection is used to produce the positive control tissue.

NEWCOMER SUPPLY VALIDATION PROCEDURE:

1. Heat dry sections in oven according to your laboratory protocol.
2. 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, if necessary, with an epitope/antigen retrieval technique approved for use in your laboratory.
4. Rinse in distilled water; tap off excess water.
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.
    a. *See Procedure Note #2.*
7. 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 *Treponema pallidum* primary antibody. Incubate at room temperature for 30 minutes.
9. Rinse slides in two changes of buffer.
10. Tap off excess buffer; apply HRP-Polymer solution. Incubate for 20 minutes.
11. Rinse slides in two changes of buffer.
12. Prepare required quantity of DAB substrate/chromogen.
13. Tap off excess buffer; apply DAB. Incubate for 5 minutes.
14. Rinse slides in four changes of distilled water.
15. Counterstain lightly with Hematoxylin Stain, Gill I (Part 1180) for 5 minutes.
16. Rinse slides in warm tap water to blue sections.
17. 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:

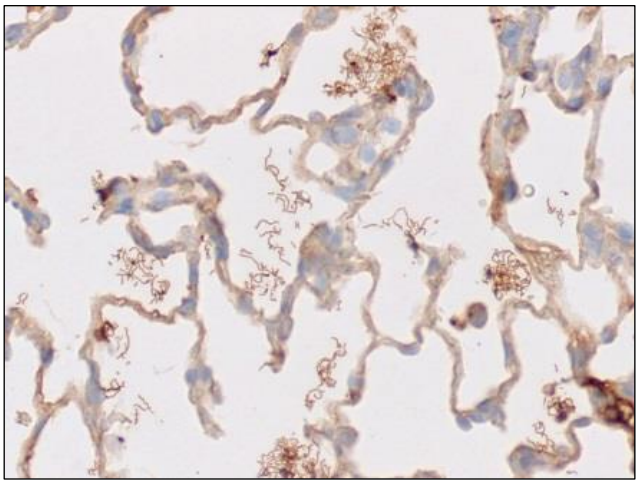
Spirochete positive expression	Brown
Negative lung	Negative for spirochetes
Nuclei	Blue

PROCEDURE NOTES:

1. Do not allow sections to dry out at any point during procedure.
2. Dilute sufficient Hydrogen Peroxide 30%, Aqueous (Part 1206) with 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.
4. Biocare *Treponema pallidum* (polyclonal ACA135) is the concentrated primary antibody used. Dilute primary antibody to 1/300 working dilution with Cell Marque Emerald: Antibody Diluent (936B).
5. Biocare Mach 2™ Universal HRP-Polymer Detection (M2U522) is the HRP-Polymer solution used.
6. Cell Marque DAB Substrate Kit (957D) is the chromogen used.
7. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

1. Biocare *Treponema pallidum* Antibody datasheet.
2. Cell Marque Emerald: Antibody Diluent datasheet.
3. Biocare Mach 2™ Universal HRP-Polymer Detection datasheet.
4. Cell Marque DAB Substrate Kit datasheet.
5. Modifications developed by Newcomer Supply Laboratory.



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