

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

<b>CONTROL SLIDES:</b>	<b>Part 3746A</b> 10 Slide/Set	<b>Part 3746B</b> 98 Slide/Set
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## **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:**

- 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.
  - See Procedure Note #1.
- Proceed, if necessary, with an epitope/antigen retrieval technique approved for use in your laboratory.
- 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.
  - See Procedure Note #2.
- Wash slides gently in distilled water. Rinse in two changes of Tris Buffered Saline.
  - See Procedure Note #3.
- Tap off excess buffer; apply *Treponema pallidum* primary antibody. Incubate at room temperature for 30 minutes.
- Rinse slides in two changes of buffer.
- Tap off excess buffer; apply HRP-Polymer solution. Incubate for 20 minutes.
- Rinse slides in two changes of buffer.
- Prepare required quantity of DAB substrate/chromogen.
- Tap off excess buffer; apply DAB. Incubate for 5 minutes.
- Rinse slides in four changes of distilled water.
- Counterstain lightly with Hematoxylin Stain, Gill I (Part 1180) for 5 minutes.
- 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:**

Spirochete positive expression	Brown
Negative lung	Negative for spirochetes
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 distilled water to a 3% (1/10) solution prior to use.
- 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.
- 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).
- Biocare Mach 2™ Universal HRP-Polymer Detection (M2U522) is the HRP-Polymer solution used.
- Cell Marque DAB Substrate Kit (957D) is the chromogen used.
- If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

## **REFERENCES:**

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

