

## Light Green SF Yellowish Counterstain for Fungus/PAS Stain - Technical Memo

### SOLUTIONS:

Light Green SF Yellowish Stain 0.1%, Aqueous

250 ml

Part 12203A

500 ml

Part 12203B

### Additionally Needed:

Fungus, PAS, *Aspergillus sp.*, Artificial Control Slides

Part 4232

or

Fungus, PAS, *Candida sp.*, Artificial Control Slides

Part 4233

Periodic Acid 0.5%, Aqueous

Part 13308

Schiff Reagent, McManus

Part 1371

Xylene, ACS

Part 1445

Alcohol, Ethyl Denatured, 100%

Part 10841

Alcohol, Ethyl Denatured, 95%

Part 10842

**For storage requirements and expiration date refer to individual product labels.**

### APPLICATION:

Newcomer Supply Light Green SF Yellowish 0.1%, Aqueous provides a complimentary counterstain for the Fungus/PAS Stain.

### METHOD:

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

**Technique:** Paraffin section cut at 4 microns

**Solutions:** All solutions are manufactured by Newcomer Supply, Inc.

All Newcomer Supply stain procedures are designed to be used with Coplin jars filled to 40 ml following the provided staining procedure.

### STAINING PROCEDURE:

1. If necessary, heat dry tissue sections/slides in oven.
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 Notes #1 and #2.
3. Place in Periodic Acid 0.5%, Aqueous (13308) for 5 minutes.
4. Wash in three changes of tap water; rinse in distilled water.
5. Drain slides of excess water and stain in Schiff Reagent, McManus (1371) for 20 minutes.
6. Wash gently in lukewarm tap water for 10 minutes to allow pink color to develop.
7. Counterstain in Light Green SF Yellowish Stain 0.1%, Aqueous for 5 seconds.
  - a. See Procedure Note #3.
8. 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:

Fungal cell walls and glycogen	Red to magenta
Background	Pale green

### PROCEDURE NOTES:

1. Drain slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during procedure.
3. Increase or decrease staining time in Light Green SF Yellowish Stain 0.1%, Aqueous for preference of counterstain intensity.
4. If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

### REFERENCES:

1. Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008. 321-323.
2. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 245.
3. Modifications developed by Newcomer Supply Laboratory.