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Fungus Stain, PAS/Light Green - Technical Memo

SOLUTIONS:	125 ml	250 ml	500 ml	1 Liter
Periodic Acid 0.5%, Aqueous		Part 13308A	Part 13308B	
Schiff Reagent, McManus	Part 1371A		Part 1371B	Part 1371C
Light Green SF Yellowish Stain 0.1%, Aqueous		Part 12203A	Part 12203B	

Additionally Needed:

Fungus, PAS, *Aspergillus sp*., Artificial Control Slides	Part 4232	or	Fungus, PAS, *Candida sp*., Artificial Control Slides	Part 4233
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 Fungus Stain, PAS/Light Green is used for identifying fungus and glycoproteins in tissue sections. Polysaccharides present in fungal cell walls are oxidized by periodic acid to aldehydes. The aldehydes then react with Schiff Reagent, McManus to yield magenta colored fungi.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin section cut at 5 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 staining procedure provided below.

STAINING PROCEDURE:

1. 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.*
2. Place slides in Periodic Acid 0.5%, Aqueous for 5 minutes.
3. Wash in three changes of tap water; rinse in distilled water.
4. Drain slides of excess water and stain in Schiff Reagent, McManus for 20 minutes.
5. Wash gently in lukewarm tap water for 10 minutes to allow pink color to develop.
6. Counterstain in Light Green SF Yellowish Stain 0.1%, Aqueous for 5 seconds.
    a. *See Procedure Note #3.*
7. 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 staining rack/slides after each step to prevent solution carry over.
2. Do not allow sections to dry out at any point during staining procedure.
3. Increase or decrease staining time in Light Green SF Yellowish Stain 0.1%, Aqueous for a length of time to suit your preference of counterstain intensity.
4. Newcomer Supply Schiff Reagent, McManus is stored at room temperature. There is no benefit to store this product at 4°C.
5. 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.