

# Alpha Amylase 1%, Aqueous for Glycogen Digestion - Technical Memo

**SOLUTION:**  
Alpha Amylase 1%, Aqueous                      500 ml  
Part 1905B

Additionally Needed:

Periodic Acid Schiff (PAS) Glycogen Control Slides	Part 4540
Periodic Acid 0.5%, Aqueous	Part 13308
Schiff Reagent, McManus	Part 1371
Hematoxylin Stain, Harris	Part 12013
Acid Alcohol 1%	Part 10011
Lithium Carbonate, Saturated Aqueous	Part 12215
Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842
Coplin Jar, Plastic	Part 5184 (for glycogen digestion microwave modification)

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

**APPLICATION:**

Newcomer Supply Alpha Amylase 1%, Aqueous is a convenient ready-to-use glycogen digestion solution for aiding in further identification of mucosubstances used in conjunction with the Periodic Acid Schiff (PAS) Stain procedure.

14. Blue in Lithium Carbonate, Saturated Aqueous (12215); 3-4 dips.
15. Wash in several changes of tap water; rinse in distilled water.
16. 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.

**METHOD:**

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

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

**RESULTS:**

Glycogen	Magenta
Glycogen digestion	Absence of magenta
Acid & neutral epithelial mucin	Magenta
Nuclei	Blue

**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. Digestion Step: See Procedure Note #3.
  - a. Two control slides and two patient slides are needed.
  - b. Label one control slide and one patient slide "with".
  - c. Label the other control slide and patient slide "without".
  - d. Place slides labeled "without" in separate Coplin jar of distilled water; hold for Step #5.
  - e. Apply Alpha Amylase 1%, Aqueous to slides labeled "with" for 30 minutes at room temperature.
  - f. Proceed to Step #5.
4. **Digestion Microwave Modification:** See Procedure Note #4.
  - a. Follow Steps #3a through #3d.
  - b. Place slides labeled "with" in a plastic Coplin jar containing Alpha Amylase 1%, Aqueous and microwave for 1 minute at 37°C. Let sit in warm solution for an additional minute.
5. Combine all slides for remaining steps; wash in running tap water for 1 minute, rinse in distilled water.
6. Place in Periodic Acid 0.5%, Aqueous (13308) for 10 minutes.
7. Wash in three changes of tap water; rinse in distilled water.
8. Place in Schiff Reagent, McManus (1371) for 20 minutes.
9. Wash in lukewarm tap water for 5 minutes.
10. Stain with Hematoxylin Stain, Harris (12013), 1-5 minutes, depending on preference of nuclear stain intensity.
11. Wash in tap water for 2-3 minutes.
12. Differentiate in Acid Alcohol 1% (10011); 1-2 quick dips.
13. Wash in tap water for 1 minute.

**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. Slides labeled "with" will be treated with amylase digestion, slides labeled "without" will not be treated for digestion.
4. The suggested microwave procedure has been tested at Newcomer Supply. This procedure is a guideline and techniques should be developed for use in your laboratory.
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. 168-171, 180.
2. Carson, Freida L., and Christa Hladik. *Histotechnology: A Self-Instructional Text*. 3rd ed. Chicago, Ill.: American Society of Clinical Pathologists, 2009. 137-141.
3. Sheehan, Dezná C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 164-168.
4. Modifications developed by Newcomer Supply Laboratory.