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Part 1905
May 2020

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

SOLUTION:	**500 ml**
Alpha Amylase 1%, Aqueous	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.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 4 microns

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: S*ee 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.
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.

RESULTS:

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

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, Dezna 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.