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Part 9162
Revised February 2016

Periodic Acid Schiff (PAS) Stain Kit - Technical Memo

KIT INCLUDES:	**Part 9162A**	**Part 9162B**
Solution A: Periodic Acid 0.5%, Aqueous	250 ml	500 ml
Solution B: Schiff Reagent, McManus	250 ml	500 ml
Solution C: Hematoxylin Stain, Harris	250 ml	500 ml
Solution D: Acid Alcohol 1%	250 ml	500 ml
Solution E: Lithium Carbonate, Saturated Aqueous	250 ml	500 ml

COMPLIMENTARY POSITIVE CONTROL SLIDES: Enclosed with this kit are two complimentary unstained positive control slides to be used for the initial verification of staining techniques and reagents. Verification must be documented by running one Newcomer Supply complimentary positive control slide along with your current positive control slide for the first run. Retain the second complimentary control slide for further troubleshooting, if needed.

Individual stain solutions and additional control slides may be available for purchase under separate part numbers at www.newcomersupply.com.

Additionally Needed:

Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842
α-Amylase	For glycogen digestion
Phosphate Buffer, pH 6.0	Part 13312 (for glycogen digestion)
Coplin Jar, Plastic	Part 5184 (for glycogen digestion microwave modification)

For storage requirements and expiration date refer to individual bottle labels.

APPLICATION:

Newcomer Supply Periodic Acid Schiff (PAS) Stain Kit procedure, with included methods for glycogen digestion, is used for staining of glycoproteins and may aid in the differential diagnosis of tumors through the detection of acid/neutral epithelial mucins and/or glycogen. Digestion steps can be employed for further identification of mucosubstances. PAS is also useful for staining basement membranes and fungal cell walls.

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 5 microns
- Cut additional control and patient slides to run digestion steps.

Solutions: All solutions manufactured by Newcomer Supply, Inc.

All Newcomer Supply Stain Kits are designed to be used with Coplin jars filled to 40 ml following the staining procedure provided below. Some solutions in the kit may contain extra volumes.

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 (page 2).*
2. Digestion Step: Proceed to Step #5 if not running Digestion.
   a. *Two control slides and two patient slides are needed. Label one control slide and one patient slide **"with"**; label the other control slide and patient slide **"without".***
   b. *Prepare Amylase Digestion Solution and mix well.*
      α-Amylase *0.05 gm*
      Phosphate Buffer, pH 6.0 (13312) 50 ml
   c. *Prepare separate Coplin jar of Phosphate Buffer, pH 6.0.*
   d. *Preheat both solutions from Steps #2b and #2c to 37°C.*
   e. *Place slides labeled **"with"** in preheated Amylase Digestion Solution and slides labeled **"without"** in preheated Phosphate Buffer, pH 6.0. Incubate both for 60 minutes at 37°C.*
   f. *Proceed to Step #4.*
3. ***Microwave Modification:** See Procedure Note #3 (page 2).*
   a. *Follow Steps #2a through #2c.*
   b. *Place slides labeled **"with"** in a plastic Coplin jar containing the Amylase Digestion Solution and slides labeled **"without"** in a plastic Coplin jar containing Phosphate Buffer, pH 6.0 and microwave both for 1 minute at 37°C.*
4. Wash all slides in running tap water for 5 minutes; rinse in distilled water. Combine slides for remaining steps.
5. Place slides in Solution A: Periodic Acid 0.5%, Aqueous for 10 minutes.
6. Wash in three changes of tap water; rinse in distilled water.
7. Place in Solution B: Schiff Reagent, McManus for 20 minutes.
8. Wash in lukewarm tap water for 5-10 minutes.
9. Stain with Solution C: Hematoxylin Stain, Harris 1-5 minutes, depending on preference of nuclear stain intensity.
10. Wash in tap water for 2-3 minutes.
11. Differentiate in Solution D: Acid Alcohol 1%; 1-2 quick dips.
12. Wash in tap water for 1 minute.
13. Blue sections in Solution E: Lithium Carbonate, Saturated Aqueous; 3-4 dips.
14. Wash in several changes of tap water; rinse in distilled water.
15. 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
Fungal cell walls	Red to purple
Basement membranes	Red to purple
Nuclei	Blue

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. The suggested microwave procedure has been tested at Newcomer Supply using an "EB Sciences", 850 watt microwave oven with temperature probe and agitation tubes. This procedure is reproducible in our laboratory. It is nonetheless a guideline and techniques should be developed for your laboratory which meet the requirements of your situation. Microwave devices should be placed in a fume hood or vented into a fume hood, according to manufacturer's instructions, to prevent exposure to chemical vapors.
4. Sigma α-Amylase from Porcine Pancreas (A3176) is the α-Amylase used in the digestion steps.
5. Newcomer Supply Schiff Reagent, McManus is stored at room temperature. There is no benefit to store this product at 4°C.
6. 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, 245.
4. Modifications developed by Newcomer Supply Laboratory.