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Part 4565
Revised January 2020

Phosphotungstic Acid Hematoxylin (PTAH) Control Slides – Technical Memo

CONTROL SLIDES:	**Part 4565A**	**Part 4565B**
	10 Slide/Set	98 Slide/Set

PRODUCT SPECIFICATIONS:
Tissue: Positive staining striated muscle.
Fixation: Formalin 10%, Phosphate Buffered (Part 1090).
Section/Glass: Paraffin sections cut at 4 microns on Superfrost™ Plus slides.
Quality Control Stain: PTAH quality control stained slide(s) included.
Reactivity: Guaranteed product specific reactivity for one year from date of receipt. Revalidate after one year to verify continued reactivity.
Storage: 15-30°C in a light deprived and humidity controlled environment.
Intended Use: To verify histological techniques and reagent reactivity.

Before using unstained control slides, review the enclosed stained slide(s) to ensure that this tissue source is acceptable for testing needs.

CONTROL SLIDE VALIDATION:

With Phosphotungstic Acid Hematoxylin (PTAH) Stain Kit:	**Part 9111A**	**Individual Stain Solution**
Solution A: Zenker Fixative, Modified, Zinc Chloride	250 ml	Part 1461
Solution B: Acetic Acid, Glacial, ACS	25 ml	Part 10010
Solution C: Potassium Permanganate 0.25%, Aqueous	250 ml	Part 133931
Solution D: Oxalic Acid 5%, Aqueous	250 ml	Part 1293
Solution E: Phosphotungstic Acid Hematoxylin (PTAH) Stain, Modified Mallory	250 ml	Part 1334

APPLICATION:

The Newcomer Supply Phosphotungstic Acid Hematoxylin (PTAH) Control Slides are for the positive histochemical demonstration of muscle striations and collagen in tissue sections.

PRESTAINING PREPARATION:

1. Heat dry sections in oven according to your laboratory protocol.
2. Prepare Zenker Fixative Working Solution; combine and mix well.
   Solution A: Zenker Fixative, Modified, Zinc Chloride 38 ml
   Solution B: Acetic Acid, Glacial, ACS 2 ml

NEWCOMER SUPPLY VALIDATION PROCEDURE:

3. 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.*
4. Fix in Zenker Fixative Working Solution (Step #2) at 56°C; 3 hours.
   Microwave Modification: *See Procedure Note #3.*
   a. *Place slides in a plastic Coplin jar containing prepared Zenker Fixative Working Solution and microwave for 5 minutes at 60°C.*
5. Wash well in three changes of tap water; rinse in distilled water.
6. Place in Solution C: Potassium Permanganate 0.25%, Aqueous for 10 minutes.
7. Wash in three changes of tap water; rinse in distilled water.
8. Place in Solution D: Oxalic Acid 5%, Aqueous for 10 minutes.
9. Wash in three changes of tap water; rinse in distilled water.
10. Stain in Solution E: PTAH Stain, Modified Mallory for 12-24 hours at room temperature, or 2 hours at 56°C.
    a. *See Procedure Note #4.*
    Microwave Modification:
    b. *Place slides in a plastic Coplin jar containing Solution E: PTAH Stain, Modified Mallory and microwave for 7 minutes at 70°C.*
11. Dehydrate quickly in two changes each of 95% and 100% ethyl alcohol. Clear in three changes of xylene, 10 dips each; coverslip with compatible mounting medium.
    a. *Dehydrate quickly, alcohol may extract stain from sections.*

RESULTS:

Muscle striations, fibrin, keratin	Dark blue
Collagen, cartilage, elastic fibers	Deep reddish brown
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. 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.
4. This PTAH Stain formulation is twice as strong as the original Mallory formulation; adjust staining time according to preference of intensity. Suggested staining time at 37°C is 18 hours.
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, 2008.130-131.
2. Carson, Freida L., and Christa Hladik Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 178-180, 201-202.
3. Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 193-194.
4. Modifications developed by Newcomer Supply Laboratory.