

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:

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

- Heat dry sections in oven according to your laboratory protocol.
- 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:

- 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.
 - See Procedure Notes #1 and #2.
- Fix in Zenker Fixative Working Solution (Step #2) at 56°C; 3 hours.
Microwave Modification: See Procedure Note #3.
 - Place slides in a plastic Coplin jar containing prepared Zenker Fixative Working Solution and microwave for 5 minutes at 60°C.
- Wash well in three changes of tap water; rinse in distilled water.
- Place in Solution C: Potassium Permanganate 0.25%, Aqueous for 10 minutes.
- Wash in three changes of tap water; rinse in distilled water.
- Place in Solution D: Oxalic Acid 5%, Aqueous for 10 minutes.
- Wash in three changes of tap water; rinse in distilled water.
- Stain in Solution E: PTAH Stain, Modified Mallory for 12-24 hours at room temperature, or 2 hours at 56°C.
 - See Procedure Note #4.
- Microwave Modification:**
 - Place slides in a plastic Coplin jar containing Solution E: PTAH Stain, Modified Mallory and microwave for 7 minutes at 70°C.
- 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.
 - 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:

- Drain slides after each step to prevent solution carry over.
- Do not allow sections to dry out at any point during procedure.
- 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.
- 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.
- If using a xylene substitute, closely follow the manufacturer's recommendations for deparaffinization and clearing steps.

REFERENCES:

- Bancroft, John D., and Marilyn Gamble. *Theory and Practice of Histological Techniques*. 6th ed. Oxford: Churchill Livingstone Elsevier, 2008.130-131.
- Carson, Freida L., and Christa Hladik Cappellano. *Histotechnology: A Self-instructional Text*. 4th ed. Chicago: ASCP Press, 2015. 178-180, 201-202.
- Sheehan, Dezna C., and Barbara B. Hrapchak. *Theory and Practice of Histotechnology*. 2nd ed. St. Louis: Mosby, 1980. 193-194.
- Modifications developed by Newcomer Supply Laboratory.

