

Phosphotungstic Acid Hematoxylin (PTAH) Control Slides – Technical Memo

<u>CONTROL SLIDES:</u>	Part 4565A	Part 4565B
	10 Slide/Set	98 Slide/Set

Phosphotungstic Acid Hematoxylin (PTAH) Control Slides contain a section of positive staining striated muscle.

PRODUCT DESCRIPTION:

The enclosed positive control slides are intended to be used to verify histological techniques and reagent reactivity. These slides are to be used for the qualitative purpose of determining positive or negative results, and are not intended to be used for any quantitative purpose. The first serial section within the control box is stained and provided for your reference. **Before using the unstained slides, review the enclosed stained slide with your pathologist to ensure that this tissue source is acceptable. Newcomer Supply will not accept a return with missing slides in the series. Newcomer Supply guarantees reactivity of these control slides for one year from the date of receipt. Revalidate after one year to verify continued reactivity. Store at 15-30°C in a light deprived and humidity controlled environment.**

These positive control slides were produced from human surgical or autopsy tissues under carefully controlled conditions. Reasonable measures are used to deliver quality control slides that are as consistent as possible. However, characteristics of quality control slides may be dissimilar due to variations in the reagents, stains, techniques, laboratory conditions, and tissue sources used. Newcomer Supply Laboratory uses a manual method of performing quality control procedures, specifically avoiding automation, in order to provide reactive control slides for even less aggressive methods of staining that our customers may be using.

CONTROL SLIDE VALIDATION:

With Phosphotungstic Acid Hematoxylin (PTAH) Stain Kit:

Solution A: Zenker Fixative, Modified, Zinc Chloride
 Solution B: Acetic Acid, Glacial, ACS
 Solution C: Potassium Permanganate 0.25%, Aqueous
 Solution D: Oxalic Acid 5%, Aqueous
 Solution E: Phosphotungstic Acid Hematoxylin (PTAH) Stain, Modified Mallory

Part 9111A	Individual Stain Solution
250 ml	Part 1461
25 ml	Part 10010
250 ml	Part 133931
250 ml	Part 1293
250 ml	Part 1334

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

APPLICATION:

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

METHOD:

Fixation: Formalin 10%, Phosphate Buffered (Part 1090)
Technique: Paraffin sections cut at 5 microns on Superfrost® Plus
Solutions: All solutions are manufactured by Newcomer Supply, Inc.

NEWCOMER SUPPLY VALIDATION 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. 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

 - a. See Procedure Note #3.
3. Fix slides in Zenker Fixative Working Solution at 56°C for 3 hours.

Microwave Modification: See Procedure Note #4 (page 2).

 - a. Place slides in a plastic Coplin jar containing prepared Zenker Fixative Working Solution and microwave for 5 minutes at 60°C.
4. Wash well in three changes of tap water; rinse in distilled water.
5. Place slides in Solution C: Potassium Permanganate 0.25%, Aqueous for 10 minutes.
6. Wash in three changes of tap water; rinse in distilled water.
7. Place slides in Solution D: Oxalic Acid 5%, Aqueous for 10 minutes.
8. Wash in three changes of tap water; rinse in distilled water.

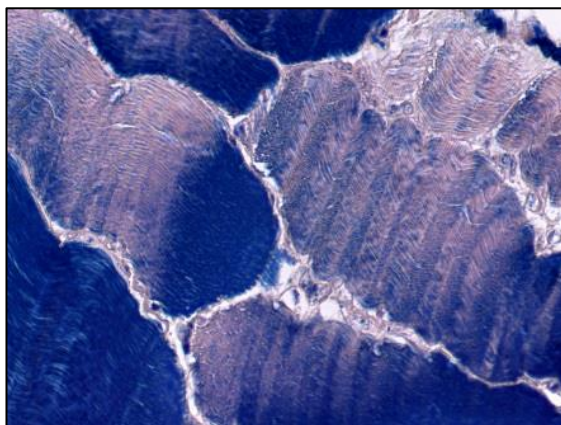
9. Place slides in Solution E: PTAH Stain, Modified Mallory for 12-24 hours at room temperature, or 2 hours at 56°C.
 - a. See Procedure Note #5 (page 2).

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.
10. 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. See Procedure Note #6 (page 2).

RESULTS:

Muscle striations, fibrin, keratin	Dark blue
Collagen, cartilage, elastic fibers	Deep reddish brown
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. Zenker fixed tissue does not require an additional Zenker fixation step. Proceed to Step #5.
4. 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.
5. Newcomer Supply PTAH Stain formula 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.
6. Dehydrate quickly as alcohol may extract stain from sections.
7. 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.