

# Phosphotungstic Acid Hematoxylin (PTAH) Stain Kit - Technical Memo

## KIT INCLUDES:

Solution A: Zenker Fixative, Modified, Zinc Chloride	<b>Part 9111A</b>
Solution B: Acetic Acid, Glacial, ACS	250 ml
Solution C: Potassium Permanganate 0.25%, Aqueous	25 ml
Solution D: Oxalic Acid 5%, Aqueous	250 ml
Solution E: Phosphotungstic Acid Hematoxylin (PTAH) Stain, Modified Mallory	250 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](http://www.newcomersupply.com).*

## Additionally Needed:

Xylene, ACS	Part 1445
Alcohol, Ethyl Denatured, 100%	Part 10841
Alcohol, Ethyl Denatured, 95%	Part 10842
Coplin Jar, Plastic	Part 5184 (for microwave modification)

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

## APPLICATION:

The Newcomer Supply Phosphotungstic Acid Hematoxylin (PTAH) Stain Kit procedure, with included microwave modification, is used for the demonstration of collagen, muscle striations and central nervous system (CNS) structures. The Zenker fixative used in this procedure is modified with zinc chloride and does not contain mercury.

## METHOD:

**Fixation:** 10% Phosphate Buffered Formalin (Part 1090)

**Technique:** Paraffin sections cut at 5 microns

**Solutions:** All solutions are 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.
2. Prepare Zenker Fixative Working Solution; combine and mix well.
 

<i>Solution A: Zenker Fixative, Modified, Zinc Chloride</i>	<i>38 ml</i>
<i>Solution B: Acetic Acid, Glacial, ACS</i>	<i>2 ml</i>

  - 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.

  - a. Place slides in a plastic Coplin jar containing 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 for 12-24 hours at room temperature, or 2 hours at 56°C.
  - a. See Procedure Note #5.

**Microwave Modification:**

  - b. Place slides in a plastic Coplin jar containing Solution E: PTAH Stain 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.

## RESULTS:

Collagen, cartilage, elastic fibers	Deep reddish brown
Muscle striations, fibrin, keratin	Dark blue
Glia fibers	Dark blue
Myelin	Lighter blue
Neurons	Salmon/Pink
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.