
MUSCLE - PHOSPHOTUNGSTIC ACID-HEMATOXYLIN, MALLORY'S (PTAH)

PURPOSE: Used for diagnosing muscle cross-striations and fibrin.

PRINCIPLE: The amount of phosphotungstic acid in the staining solution is far greater than the amount of hematein and it is believed that tungsten binds all available components blue, while the phosphotungstic acid is thought to stain the red-brown components. This stain has been referred to as a polychrome stain because one solution gives two major colors. The components colored red-brown will lose this color with water or prolonged alcohol washes, and dehydration of the section following staining therefore must be rapid.

CONTROL: Use skeletal or cardiac muscle, tissue containing fibrin for fibrin staining, and cerebral cortex for glial fibers.

FIXATIVE: Zenker's fixative preferred or formalin fixed.

TECHNIQUE: Cut paraffin sections 4m for muscle, 6m for glial fibers.

EQUIPMENT: Coplin jars, microwave oven. Rinse all glassware in DI water.

REAGENTS:

Lugol's:

See Stock Solutions

5% Hypo:

See Stock Solutions

PTAH Stain:

Purchased through American
Histology Reagent Company

CAUTION: Avoid contact and inhalation.

0.25% Potassium Permanganate:

Potassium permanganate 0.25 gm
Distilled water 100.0 ml

Mix fresh, discard after use.

CAUTION: Strong oxidant.

5% Oxalic Acid Solution:

Oxalic acid 5.0 gm
Distilled water 100.0 ml

Mix well, label with initial and date. Solution is stable for 1 year.

CAUTION: avoid contact and inhalation

CONNECTIVE TISSUE

PTAH

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Zenker's Fluid:

Distilled water	500.0 ml
Mercuric chloride	25.0 gm
Potassium dichromate	12.5 gm
Sodium sulfate	5.0 gm

Mix well, label with date and initial. Solution is stable indefinitely.

Working Solution:

Right before use, to 50 ml of Zenker's add 5 ml glacial acetic acid. Discard after use.

CAUTION: Corrosive poison, discard in labeled glass container.

SAFETY: Wear nitrile gloves and lab coat, wear a particle mask or work under the hood. Keep all hot uncapped solutions under the hood. Avoid contact and inhalation.

Zenker's contains; Mercuric chloride: Severe skin and eye irritant; target organ effects on reproductive, urogenital, respiratory, gastrointestinal and fetal systems via ingestion and inhalation routes.

Potassium dichromate: toxic by inhalation of dust and by ingestion; target organ effects on reproductive and fetal systems. Solid form is corrosive to eyes, skin and mucous membranes.

Iodine: dermal sensitizer, irritant to eyes, skin and respiratory system.

Sodium thiosulfate: Toxic on ingestion. Can irritate the stomach. Irritant to skin, eyes and respiratory tract.

Potassium permanganate: Skin and eye irritant, ingestion will lead to severe gastrointestinal distress. Strong oxidant.

Oxalic acid: can cause severe burns of the eyes, skin or mucous membranes. Toxic by inhalation and ingestion. Target organ effects on kidneys and cardiovascular system, repeated exposure can cause dermatitis. Corrosive.

PROCEDURE:

1. Deparaffinize and hydrate to distilled water.
2. *Zenker's fixative, microwave Hi power, 45 seconds, allow to stand for 5 minutes.
3. Wash with tap water.
4. Lugol's iodine for 5 minutes.
5. Wash in tap water.
6. 5% hypo for 1-2 minutes.(for glial fibers, bleach out iodine in 95% alcohol in place of hypo.)
7. Wash in tap water 10 minutes.
8. Oxidize in 0.25% potassium permanganate, 5 minutes.
9. Wash in tap water.
10. Bleach in 5% oxalic acid until white.
11. Wash in tap water, rinse in distilled water.
12. PTAH stain, overnight, room temperature.
13. Dehydrate rapidly through two changes each of 95%, 100% alcohol, and two changes of xylene, coverslip.

RESULTS:

Cross-striations, fibrin, glial fibers	blue
Nuclei	blue
Collagen	red-brown
Elastic fibers	purplish

REFERENCES:

- Sheehan D, Hrapchak B, Theory and practice of Histotechnology, 2nd Ed, 1980, pp188,192-193, Battelle Press, Ohio
- Carson, F, Histotechnology: A Self-Instructional Text, 1990, pp156-157, ASCP,III
- Crookham,J, Dapson,R, Hazardous Chemicals in the Histopathology Laboratory, 2nd ED, 1991, Anatech

Prepared: _____ By: _____

Approved: _____ By: _____

PROCEDURE CARD

PTAH-PHOSPHOTUNGSTIC ACID-HEMATOXYLIN, MALLORY'S

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RESULTS:

Cross-striations, fibrin, glial fibers, nuclei: blue
Collagen: red-brown
Elastic fibers: purplish

Zenker's Fixative:

SAFETY: Wear gloves and lab coat, work under the hood or in a well ventilated room. Do not discard Zenker's fix down the drain, it contains mercury. Dispose through Safety Services.

Lugol's and 5% Hypo:

See: Stock Solutions

PTAH Stain:

Purchased through American Histology Reagent Company

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Potassium permanganate	0.25 gm
Distilled water	100.0 ml

Mix fresh, discard after use.

5% Oxalic Acid Solution:

Oxalic acid	5.0 gm
Distilled water	100.0 ml

Mix well, label with initial and date. Solution is stable for 1 year.

5% OXALIC ACID

Oxalic acid 10.0 gm

Distilled water 200.0 ml

Solution is stable for 1 year.

CAUTION: Avoid contact and inhalation.

TECH: _____

DATE: _____

EXPIRATION: _____

1% Oxalic Acid

TECH: _____

DATE:

PTAH Hematoxylin

TECH: _____

DATE: _____