Version 4 Last updated 16 April 2020

ab150683 Phosphotungstic Acid Hematoxylin (PTAH) Stain Kit (Connective Tissue Stain)

For the histological visualization of collagen, striated muscle and glial fibers.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview 1
2. Materials Supplied and Storage 2
3. Materials Required, Not Supplied 3
4. General guidelines, precautions, and troubleshooting 4
5. Staining Procedure for paraffin-embedded sections 5
6. Staining Procedure for frozen sections 6
7. FAQs / Troubleshooting 7
8. Notes 8

1. Overview

The Phosphotungstic Acid Hematoxylin (PTAH) Stain Kit (Connective Tissue Stain) is intended for use in the histological visualization of striated muscle, glial fibers and collagen without using Zenker's Fixative containing mercuric chloride.

This kit may be used on formalin-fixed, paraffin-embedded or frozen sections.

Staining Interpretation:

Fibrin, striated muscle, glial fibers	Blue
Collagen	Brown/Red
Nuclei	Blue

Control Tissue:

Striated muscle.

2. Materials Supplied and Storage

Store kit at Room temperature immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Keep away from open flame and refer to the safety datasheet.

Item	Quantity	Storage temperature (before prep)
Zinc Chloride Solution (10%)	500 ml	RT
Ferric Ammonium Sulfate	125 ml	RT
PTAH Solution	125 ml	RT

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

Paraffin-embedded sections:

- 500-watt microwave oven
- 95% alcohol
- Absolute alcohol
- Xylene or xylene substitute

Frozen sections:

- Water bath, prewarmed at 60 °C
- 95% alcohol
- Absolute alcohol
- Xylene or xylene substitute

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Staining Procedure for paraffin-embedded sections

– Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.

5.1 Deparaffinize sections and hydrate in distilled water.
5.2 Place slide in fresh distilled water for 1 minute.
5.3 Pour 50 ml of Zinc Chloride Solution into a plastic coplin jar and heat in the microwave for 20 seconds on high power.
5.4 Remove jar and stir the solution to equalize the temperature.
5.5 Return the jar to the microwave and heat on high power for a further 10 seconds. Stir the solution again.
5.6 Place the slide in the coplin jar and incubate for 15 minutes.
5.7 Rinse slide in running tap water for 1 minute.
5.8 Rinse in distilled water for 1 minute.
5.9 Place slide in 25 ml Ferric Ammonium Sulfate aqueous solution, heat in microwave for 15 seconds on high power and incubate for 2 minutes.
5.10 Rinse slide in running tap water for 2 minutes.
5.11 Rinse in distilled water for 1 minute.
5.12 Heat 25 ml PTAH Solution in the microwave for 20 seconds on high power.
5.13 Remove and agitate to equalize the temperature.
5.14 Place the slide in the stain, agitate and incubate for 15 minutes.
5.15 Reheat the solution for a further 10 seconds on high power, agitate and incubate the slide for another 15 minutes.
5.16 Differentiate the section in 95% alcohol. Check using a microscope for proper differentiation.
5.17 Dehydrate in 3 changes of absolute alcohol.
5.18 Clear in 3 changes of xylene or xylene substitute and mount in synthetic resin.

6. Staining Procedure for frozen sections

- Equilibrate all materials and prepared reagents to room temperature just prior to use.

6.1 Hydrate sections in distilled water.
6.2 Pour 50 ml of Zinc Chloride Solution into a plastic coplin jar and set in 60 °C water bath for 10 minutes to equilibrate temperature.
6.3 Place slide in warmed Zinc Chloride Solution and incubate for 20 minutes at 60 °C.
6.4 During step 6.3, pour Ferric Ammonium Sulfate Solution into a second plastic staining jar and place in 60 °C water bath for 10 minutes to equilibrate temperature.
6.5 Rinse slide in running tap water for 1 minute.
6.6 Rinse slide in distilled water for 1 minute.
6.7 Place slide in warmed Ferric Ammonium Sulfate Solutio and incubate for 5 minutes at 60 °C.
6.8 During step 6.7, pour PTAH Solution into a third plastic staining jar and place in 60 °C water bath for 10 minutes to equilibrate temperature.
6.9 Rinse slide in running tap water for 2 minutes.
6.10 Rinse slide in distilled water for 1 minute.
6.11 Place slide in warmed PTAH Solution and incubate for 60 minutes at 60 °C.
6.12 Differentiate sections in 95% Reagent Alcohol. Check section using microscope for proper differentiation.

Note: Graded alcohols will remove some stain.

6.13 Dehydrate in 3 changes of Absolute Alcohol.
6.14 Clear in 3 changes of fresh Xylene or Xylene Substitute, and mount in synthetic resin.

7. FAQs / Troubleshooting

Problem	Solution
The differentiation was not successful	• Reduce the original incubation time or temperature of the dye solution • Prepare thinner sections, usually 3 -5 µm

General troubleshooting points are found at www.abcam.com/assaykitguidelines

8. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)