Diagnostic BioSystems

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PTAH Stain Kit
(Phosphotungstic Acid Hematoxylin)

Catalog Number: KT029

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Intended Use
For In Vitro Diagnostic Use

Summary and Explanation
The PTAH Stain Kit is intended for use in the histological visualization of collagen, 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.

Fibrin, Striated Muscle, Glial Fibers: Blue
Collagen: Brown/Red
Nuclei: Blue

Control Tissue
Tissues fixed in 10% formalin are suitable for use prior to paraffin embedding. Consult references (Kiernan, 1981: Sheehan & Hrapchak, 1980) for further details on specimen preparation.

1. Cut sections, usually 3 to 5 µm and pick the sections up on glass slides.
2. Bake the slides for at least 30 minutes at approximately 70°C.
3. Allow to cool.

Recommended Positive Control
1. Striated Muscle

Reagents Provided

Kit Contents	Volume	Storage
Zinc Chloride Solution (10%)	500 mL	15-30°C
Ferric Ammonium Sulfate	125 mL	15-30°C
PTAH Solution	125 mL	15-30°C

Storage and Handling
Do not use product after the expiration date printed on vial. If reagents are stored under conditions other than those specified here, they must be verified by the user. Diluted reagents should be used promptly.

Staining Procedure (Water Bath @ 60°C)
Equipment Needed: Heated Water Bath

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Pour Zinc Chloride Solution (10%) into plastic staining jar and set in 60° C. water bath for 10 minutes to equilibrate temperature.
3. Place slide in warmed Zinc Chloride Solution (10%) and incubate for 20 minutes at 60°C.
4. During step 3, pour Ferric Ammonium Sulfate Aqueous Solution into a second plastic staining jar and set in 60° C. water bath for 10 minutes to equilibrate temperature.
5. Rinse slide in running tap water for 1 minute.
6. Rinse in distilled water for 1 minute.
7. Place slide in warmed Ferric Ammonium Sulfate Aqueous Solution and incubate for 5 minutes at 60°C.
8. During step 7, pour Phosphotungstic Acid Hematoxylin Solution into a third plastic staining jar and set in 60°C water bath for 10 minutes to equilibrate temperature.
9. Rinse slide in running tap water for 2 minutes.
10. Rinse in distilled water for 1 minute.
11. Place slide in warmed Phosphotungstic Acid Hematoxylin Solution and incubate for 60 minutes at 60°C.
12. Differentiate section in 95% Reagent Alcohol. Check section using microscope for proper differentiation.
13. Note: Graded alcohols will remove some stain.
14. Dehydrate in 3 changes of Absolute Alcohol.
15. Clear in 3 changes of fresh Xylene or Xylene Substitute, and mount in synthetic resin.

Staining Procedure (Microwave)
Equipment Needed: 500 Watt Microwave Oven

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Place slide to fresh distilled water for 1 minute.
3. Pour 50 ml of Zinc Chloride Solution (10%) into plastic coplin jar and heat in microwave for 20 seconds on high power. Remove jar and stir solution to equalize temperature. Return coplin jar to microwave and heat for 10 seconds on high power. Remove jar and stir solution to equalize temperature.
4. Place slide in coplin jar and incubate for 15 minutes.
5. Rinse slide in running tap water for 1 minute.
6. Rinse in distilled water for 1 minute.
7. Place slide in 25 ml Ferric Ammonium Sulfate Aqueous Solution, heat in microwave for 15 seconds on high power and incubate for 2 minutes.
8. Rinse slide in running tap water for 2 minutes.
9. Rinse in distilled water for 1 minute.
10. Heat 25 ml Phosphotungstic Acid Hematoxylin Solution in microwave for 20 seconds on high power. Remove and agitate to equalize temperature of solution. Place slide in stain, agitate and incubate for 15 minutes. Reheat solution for 10 seconds on high power, agitate and incubate for another 15 minutes.
11. Differentiate section in 95% Reagent Alcohol. Check section using microscope for proper differentiation.
12. Dehydrate in 3 changes of Absolute Alcohol.
13. Clear in 3 changes of fresh Xylene or Xylene Substitute, and mount in synthetic resin.

Limitations of the Procedure

1. Histological staining is a multiple step diagnostic process that requires specialized training in the selection of the appropriate reagents, tissue selections, fixation, processing, preparation of the slide, and interpretation of the staining results.
2. Tissue staining is dependent on the handling and processing of the tissue prior to staining.
3. Improper fixation, freezing, thawing, washing, drying, heating, sectioning, or contamination with other tissues or fluids may produce artifacts or false negative results.
4. The clinical interpretation of any positive staining, or its absence, must be evaluated within the context of clinical history, morphology and other histopathological criteria. It is the responsibility of a qualified pathologist to be familiar with the special stain and methods used to produce the slide.
5. Staining must be performed in a certified licensed laboratory under

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the supervision of a pathologist who is responsible for reviewing the stained slides and assuring the adequacy of positive and negative controls.

Precautions

1. Consult local and/or state authorities with regard to recommended method of disposal.
2. Materials of human or animal origin should be handled as biohazardous materials and disposed of with proper precautions.
3. Avoid microbial contamination of reagents. Contamination could produce erroneous results.
4. This reagent may cause irritation. Avoid contact with eyes and mucous membranes.
5. If reagent contacts these areas, rinse with copious amounts of water.
6. Do not ingest or inhale any reagents.
7. Use in a chemical fume hood whenever possible.

Troubleshooting

If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem is suspected, contact Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com.

References

I. Shapiro, S.H., Sohn, L.C.; Rapid Microwave Phosphotungstic Acid-Hematoxylin Stain for Paraffin and Glycol Methacrylate Sections; The Journal of Histotechnology; Volume 17, Number 2, June 1994, pages 125-126.