Diagnostic BioSystems

Instructions For Use

KT 029-IFU

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6616 Owens Drive, Pleasanton, CA 94588 U.S.A. - Tel. (925) 484-3350 - Fax (925) 484-3390 - www.dbiosys.com

PTAH Stain Kit

(Phosphotungstic Acid Hematoxylin)

Description and Principle

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 with Mercuric Chloride as a mordant. This kit may be used on formalin-fixed, paraffin-embedded sections.

Zinc chloride acts to mordant the tissue for trichrome staining and is a safe and effective replacement for traditionally used mercuric chloride treatment. Aldehydes are formed by the oxidizing action of ferric ammonium sulfate. Hematoxylin and phosphotungstic form a blue dye lake staining fibrin, muscle, glial fibers, and nuclei. Collagen is dominantly stained by phosphotungstic acid resulting in lighter orange, salmon, or red/brown

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Expected Results

Fibrin, Striated Muscle, Glial Fibers:	Blue to Purple
Collagen:	Light Orange/Salmon to Brownish/Red
Nuclei:	Blue to Purple

Kit Contents	Storage
1. Zinc Chloride Solution (10%)	15-30° C
2. Ferric Ammonium Sulfate	15-30° C
3. PTAH Solution	15-30° C

Suggested Controls *(not provided)*

Striated Muscle

Uses/Limitations

For In-Vitro Diagnostic use only.
Do not use if reagents become cloudy or precipitate
Do not use past expiration date.
Use caution when handling reagents.
Non-Sterile
Intended for FFPE sections cut at 5-10µm.
This procedure has not been optimized for frozen sections.
Frozen sections may require protocol modification.

Storage

Store kit and all components at room temperature (15-30°C).

Safety and Precautions

Please see current Safety Data Sheets (SDS) for this product and components GHS classification, pictograms, and full hazard/precautionary statements. If there is any serious incident that has occurred in relation to the device, please contact the manufacturer: Diagnostic BioSystems Technical Support at (925) 484-3350, extension 2 or techsupport@dbiosys.com. If required, please report to the Competent Authority of the Member State in which the user and/or patient is established.

Procedure (Standard):

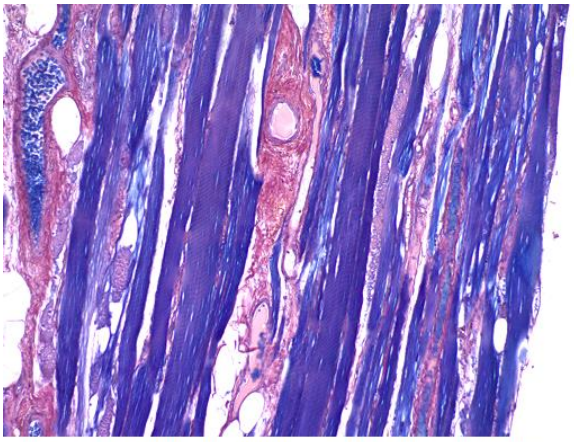
1. Deparaffinize sections if necessary and hydrate to distilled water.

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.



Human Skeletal Muscle and Collagen at 10X stained with the PTAH Stain Kit (Phosphotungstic Acid Hematoxylin)

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.

Note: Graded alcohols will remove some stain.

13. Dehydrate in 3 changes of Absolute Alcohol.

14. Clear in 3 changes of fresh Xylene or Xylene Substitute, and mount in synthetic resin.

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Tel: (925) 484 3350
www.dbiosys.com

CE IVD

CH REP
MedEnvoy Switzerland
Gotthardstrasse 28
6302 Zug
Switzerland

EC REP
MedEnvoy Global B.V.
Prinses Margrietplantsoen 33 - Suite 123
2595 AM The Hague
The Netherlands

Procedure (Dropper):
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. Fill a plastic staining jar with DI water. Place in microwave and heat until hot but not boiling.

4. Carefully lay slide across the top of the staining jar and apply 5 drops of Zinc Chloride Solution (10%). Heat in microwave for 10 seconds. Leave jar with slide in the microwave during the incubation period to maintain temperature.

5. Incubate slide for 15 minutes. **Note:** Water in staining jar will maintain reagent temperature during staining procedure.

6. Rinse slide in running tap water for 1 minute.

7. Rinse in distilled water for 1 minute.
Note: During rinse step, reheat water in staining jar to hot but not boiling.

8. Carefully lay slide across the top of the staining jar and apply 5 drops of Ferric Ammonium Sulfate Solution and heat in microwabe for 10 seconds. Leave jar with slide in the microwave during the incubation period to better maintain temperature.

9. Incubate slide for 2 minutes.

10. Rinse slide in running tap water for 2 minutes.

11. Rinse in distilled water for 1 minute.
Note: During rinse step, reheat water in staining jar to hot but not boiling.

12. Carefully lay slide across the top of the staining jar and apply 5 drops of Phosphotungstic Acid Hematoxylin Solution and heat in microwave for 10 seconds. Leave jar with slide in the microwave during the incubation period to better maintain temperature.

13. Incubate slide for 15 minutes.

14. Shake off Phosphotungstic Acid Hematoxylin Solution and repeat steps 13 and 14.

15. Differentiate section in 95% Reagent Alcohol. Check section using microscope for proper differentiation.

16. Dehydrate in 3 changes of Absolute Alcohol.

17. Clear in 3 changes of fresh Xylene or Xylene Substitute and mount in synthetic resin.

References
1. 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.

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