ScyTek LABORATORIES INC.

Instructions For Use

TIM-IFU

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Trichrome Stain Kit (with Iodine)

Description and Principle

The Trichrome Stain Kit (with Iodine) is intended for use in the histological visualization and differentiation of collagen in tissue sections. Gram's Iodine is offered as an alternative to Bouin's Fluid post fixative and is significantly less hazardous. Iodine post-fixation improves the color and staining of collagen and is comparable to trichrome kits that utilize Bouin's. No nuclear counterstain is included with this kit. This kit is intended for use on formalin-fixed sections.

Sections are first mordanted with Gram's iodine which acts to intensity subsequent trichrome staining. Biebrich Scarlet / Acid Fuchsin Solution stains all acidophilic tissue elements red. Phosphotungstic aicd displaces the red dye out of collagen fibers leaving them colorless and receptive to staining by aniline blue.

Expected Results

Collagen:	Blue
Muscle Fibers:	Red
Nuclei:	Red

Kit Contents

	Storage
1. Gram's Iodine	18-25°C
2. Biebrich Scarlet / Acid Fuchsin Solution	18-25°C
3. Phosphotungstic Acid Differentiator	18-25°C
4. Aniline Blue Solution	18-25°C
5. Acetic Acid Solution (1%)	18-25°C

Suggested Controls *(not provided)*

Lung, Liver, Colon, Stomach

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 all components at room temperature (18-25°C).

Safety and Precautions

Please see current Safety Data Sheets (SDS) for this product and components GHS classification, pictograms, and full hazard/precautionary statements.

Procedure:

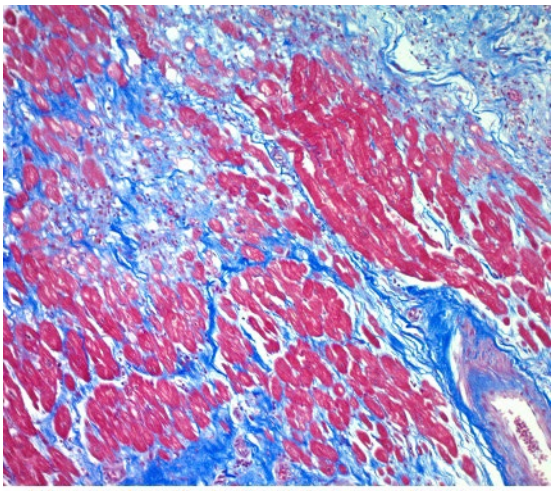
1. Deparaffinize sections if necessary and hydrate to distilled water.

2. Preheat Gram's Iodine in a water bath to 56° - 64° centigrade in a fume hood or very well ventilated area.

3. Place slides in preheated Gram's Iodine for 60 minutes followed by a 10 minute cooling period.

4. Rinse slide in tap water for at least 10 minutes.

5. Rinse slide in distilled water.



Trichrome Stain Kit with Gram's Iodine post-fixative on a human heart that had suffered a heart attack.

6. Apply Biebrich Scarlet / Acid Fuchsin Solution to slide for 5-10 minutes.

7. Rinse slide in distilled water.

8. Differentiate in Phosphotungstic Acid Differentiator for 15-30 seconds.
Note: Do not exceed 30 seconds to avoid over-differentiation. Check microscopically, if sections are over-differentiated, rinse in distilled water and repeat steps 6-7.

9. Without rinsing, apply Aniline Blue Solution to slide for 3-5 minutes.

10. Rinse slide in distilled water.

11. Apply Acetic Acid Solution (1%) to slide for 3-5 minutes.

12. Dehydrate in absolute alcohol.

13. Clear in Xylene or Xylene Substitute, and mount in synthetic resin.

Note: Bouin's Fluid (not provided) may be needed to achieve the very bright red colors sometimes associated with trichrome staining.

References

1 Yu, Yufeng, and Clifford M. Chapman. "Masson trichrome stain: Postfixation substitutes." Journal of histotechnology 26.2 (2003): 131-134.
2. A.F.I.P. Laboratory Methods in Histotechnology; 1992, pages 132-133.
3. Bekeredjian R., Walton C.B., MacCannell K.A., Ecker J., Kruse F., et al. Conditional HIF-1α Expression Produces a Reversible Cardiomyopathy. PLoS ONE 5(7): e11693. Doi:10.1371/journal.pone.0011693. (2010)
4. Zwaans BM, Krueger S, Bartolone SN, Chancellor MB, Marples B, Lamb LE. Modeling of chronic radiation-induced cystitis in mice. Advances in Radiation Oncology. 2016 Aug 1.
5. Sheehan, DC., Hrapchak, BB. Theory and Practice of Histotechnology; 1980, page 190.

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