CANCER DIAGNOSTICS, INC.

Instructions For Use

SS1056-125

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Martius Scarlet Blue (MSB) Stain Kit

Description and Principle

The Martius Scarlet Blue (MSB) stain kit is intended for use in the histological demonstration of fibrin, muscle, collagen, and erythrocytes. MSB is a reliable method of differentiating fresh and old fibrin clusters.
Martius Yellow, a small-molecule dye selectively stains erythrocytes and early fibrin deposits. A medium-sized molecule dye, Crystal Scarlet, and larger molecule dyes, Phosphotungstic Acid and Aniline Blue, are employed in a trichrome-type staining giving red fibrin and muscle. Collagen and older fibrin clusters are stained blue.

Expected Results

Fibrin:	Red (fresh fibrin may stain yellow and old fibrin blue)
Muscle:	Pink to Pale Red
Erythrocytes:	Yellow
Nuclei:	Red to Black
Collagen:	Blue
Other Connective Tissue:	Blue

Kit Contents

	Storage
1. Bouin's Fluid	18-25 C
2. Weigert's Iron, Hematoxylin (A)	18-25 C
3. Weigert's Iron, Hematoxylin (B)	18-25 C
4. Martius Yellow Solution	18-25 C
5. Crystal Scarlet Solution	18-25 C
6. Phosphotungstic Acid Differentiator	18-25 C
7. Aniline Blue Solution (MSB)	18-25 C

Suggested Controls *(not provided)*

Placenta, Artery, Kidney,

Uses/Limitations

For Research 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

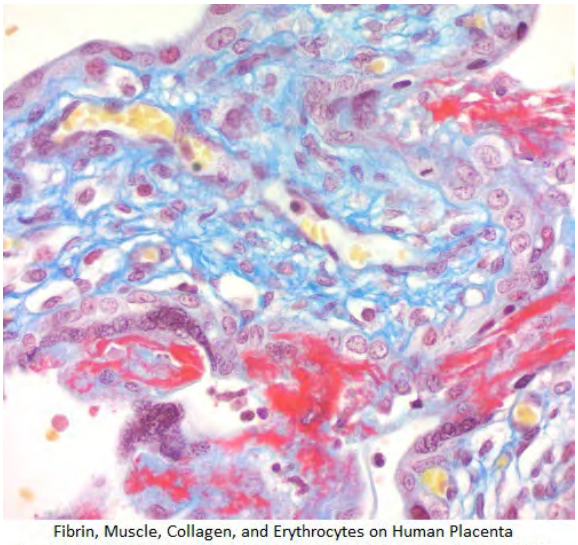
Store kit and all components at room temperature (18-25 C).

Safety and Precautions

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

Optimization Notes

1.Incomplete formalin fixation and/or Bouin's post-fixation can cause poor staining of erythrocytes resulting in red or orange instead of expected yellow staining. Best results are obtained through overnight fixation in mercury or zinc fixatives followed by overnight paraffin processing. Formalin fixed, paraffin embedded sections also gives acceptable results if refixed in Bouin's Fluid for at least 1 hour at 56° - 64° centigrade.



Fibrin, Muscle, Collagen, and Erythrocytes on Human Placenta demonstrated with Martius Scarlet Blue Stain Kit (MSB-1) viewed at 40X

Procedure

1. Deparaffinize sections and hydrate to distilled water.

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

3. Place slides in preheated Bouin's Fluid for 60 minutes followed by a 10-minute cooling period. Increase post-fixation time as needed.

4. Rinse slides in running tap until sections are completely clear.

5. Mix equal parts of Weigert's (A) and Weigert's (B) and stain slide with working Weigert's Iron Hematoxylin for 2-5 minutes.

6. Rinse slides in running tap water for 2 minutes.

7. Rinse slides with distilled water.

8. Apply Martius Yellow Solution for 2-3 minutes.

9. Rinse slides with distilled water

10. Apply Crystal Scarlet Solution for 2-5 minutes.

11. Rinse slides with distilled water.

12. Differentiate in Phosphotungstic Acid Differentiator for 2-10 minutes. Check microscopically and continue differentiation if needed.

13. Rinse slides with distilled water.

14. Apply Aniline Blue Solution (MSB) for 2-5 minutes. Check microscopically until collagen is the desired intensity of blue.

15. Dehydrate rapidly in three changes of absolute alcohol.

16. Clear in xylene or substitute and mount in synthetic resin.

References

1. Bancroft, J. D., and A. Stevens. "Theory and Practice of Histo-logical Techniques ed 2 Churchill-Livingstone." *Edinburgh and London, UK* (1982).
2. Lendrum, A. C., D. S. Fraser, W. Slidders, and R. Henderson. "Studies on the character and staining of fibrin." *Journal of clinical pathology* 15, no. 5 (1962): 401.