



Instructions For Use

HSK-IFU

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Herovici Stain Kit

Description and Principle

The Herovici stain kit is intended to differentiate between young and mature collagen. Young collagen and reticulin stain blue and Mature collagen stains red. Weigert's Iron Hematoxylin is provided to stain nuclei blue to black.

Herovici stain kit utilizes a combination of dyes used in a polychromatic type of staining. Young collagen and reticulin, and mature collagen are differentially stained according to their affinity for the red and blue dyes. Erythrocytes are stained by a yellow counterstain.

Expected Results

Mature collagen:	Red
Young collagen and reticulin:	Blue
Nuclei:	Blue to Black
Erythrocytes:	Yellow to Grey

Kit Contents

<u>Kit Contents</u>	<u>Storage</u>
1. Herovici Solution A	18-25°C.
2. Herovici Solution B	18-25°C.
3. Hematoxylin Weigert's Iron A	18-25°C.
4. Hematoxylin Weigert's Iron B	18-25°C.

Suggested Controls (not provided)

Skin.

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

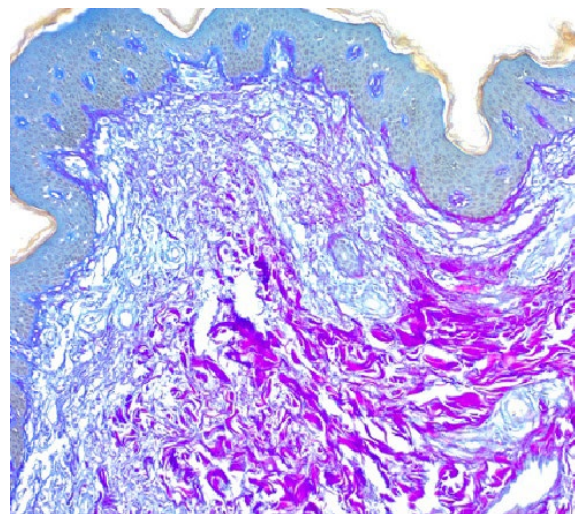
Store kit and all components at room temperature (18-25°C).

Safety and Precautions

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

Procedure:

1. Deparaffinize and hydrate to water through graded alcohols.
2. Mix equal parts (1:1) Weigert's Iron parts A and B. Use immediately and dispose of after use (do not re-use). Apply to tissue and for 5-10 minutes.
3. Rinse slide in tap water for at least 2 minutes. Then rinse briefly in deionized water.
4. Mix equal parts (1:1) Herovici Solutions parts A and B. Use immediately and dispose of after use (do not re-use). Apply to tissue and stain for 2 minutes.
5. Rinse slide briefly with absolute alcohol followed by dehydration in absolute alcohol.



Herovici stain on Normal Human Skin demonstrating young and mature collagen and reticulin fibers

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

Important notes.

1. Blue stain leaches from slide over time. If running comparison experiments run within the same day or week if possible.

2. The Herovici's working solution (step 4) contains multiple dyes and there several factors that may contribute to staining balance such as tissue quality and fixation. If staining balance needs to be altered, it may be controlled by adjusting the ratio of the mixed working solution. For example, if smaller, younger fibers are staining red, the solution may be altered to 60% blue dye (HVA) to 40% red dye (HVB) (instead of 1:1) to increase blue coloration. A control slide that gives consistent results (e.g. skin) should be run alongside experimental slides to verify consistency.

References

1. Oh, S.; Rho, N.-K.; Byun, K.-A.; Yang, J.Y.; Sun, H.J.; Jang, M.; Kang, D.; Son, K.H.; Byun, K. Combined Treatment of Monopolar and Bipolar Radiofrequency Increases Skin Elasticity by Decreasing the Accumulation of Advanced Glycated End Products in Aged Animal Skin. *Int. J. Mol. Sci.* 2022, 23, 2993. <https://doi.org/10.3390/ijms23062993>
2. (1963) Methyl Methacrylate Monomer; A Solvent for Removal of Implanted Electrodes, *Stain Technology*, 38:3, 201-206, DOI: 10.3109/10520296309067167



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