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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 



Instructions For Use

MPS-IFU

205 South 600 West Logan, Utah 84323, U.S.A. – Tel. (800) 729-8350 – Tel. (435) 755-9848 – Fax (435) 755-0015 – www.scytek.com Rev. 6, 2/2/2021

Movat Pentachrome Stain Kit

(Modified Russell-Movat)

Description and Principle

This Movat Pentachrome Stain Kit (Modified Russell-Movat) is intended for use in the histological demonstration of collagen, elastin, muscle, and mucin in FFPE sections. This procedure has historically been particularly useful when studying the heart, blood vessels and various vascular diseases.

Entire tissue is initially overstained with a Verhoeff-type working Elastic Stain Solution which contains unoxidized Hematoxylin, an oxidizer (Ferric Chloride) and a mordant (Iodine). Excess elastin stain is then removed from tissue using a dilute Ferric Chloride solution that differentiates elastin and nuclei (black) from the rest of the tissue. Alcian blue, a copper phthalocyanine dye, is then used to bind acid mucins providing bright blue color. A trichrome-type of staining using Biebrich Scarlet/Acid, Phosphotungstic Acid, and a proprietary Yellow Stain Solution, is then employed giving red muscle and yellow collagen.

Expected Results

Elastic Fibers:	Black
Nuclei:	Blue/Black to Red
Collagen:	Yellow to Red
Mucin:	Bright Blue
Muscle:	Red

Kit Contents

Kit Contents	Storage
1. Hematoxylin Solution (5%)	18-25°C
2. Ferric Chloride Solution (10%)	18-25°C
3. Lugol's Iodine Solution	18-25°C
4. Ferric Chloride Solution (2%)	18-25°C
5. Sodium Thiosulfate Solution (5%)	18-25°C
6. Acetic Acid Solution (1%)	18-25°C
7. Alcian Blue Solution, pH 2.5	18-25°C
8. Biebrich Scarlet – Acid Fuchsin Solution	18-25°C
9. Phosphotungstic Acid Solution (5%)	18-25°C
10. Yellow Stain Solution	18-25°C

Suggested Controls (not provided)

Heart, Lung, Skin, GI Tract

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 (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.

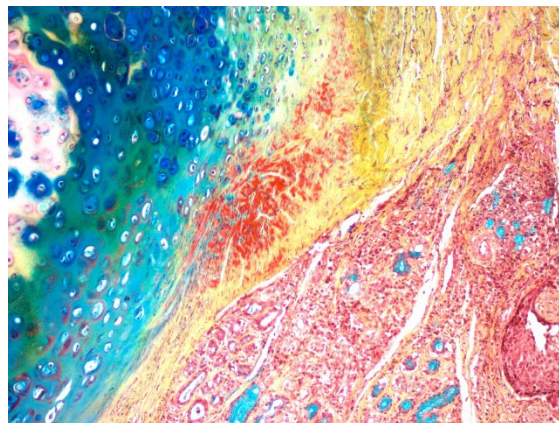


Figure 1 Movat Pentachrome staining on human Lung

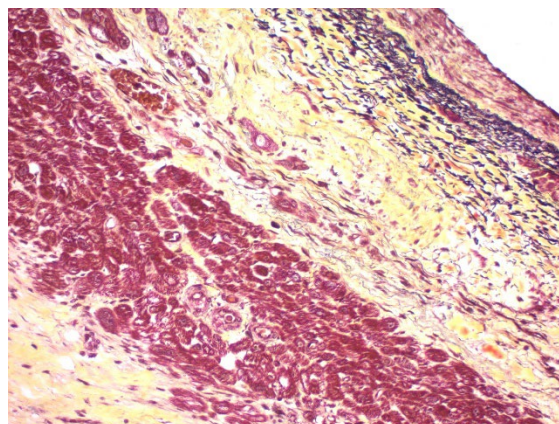


Figure 2. Movat Pentachrome staining on human Heart.

Preparation of Reagents Prior to Beginning:

Prepare **Working Elastic Stain Solution** by mixing:

- 2 parts Hematoxylin Solution (5%)
- 1 part Ferric Chloride Solution (10%)
- 1 part Lugol's Iodine Solution.

(mixed solution may be used for 24 hours)

Example: 2mLs Hematoxylin solution, 1mL Ferric Chloride, 1mL Lugol's Iodine.

Example (dropper): Use enclosed graduated mixing vial - 14 drops (560µl) + 7 drops (280µl) + 7 drops (280µl) Total: 1120µl or 1.12ml (1 drop = ~40µl)

We suggest making at least 1mL working solution per slide if staining on horizontal slides because solution is alcoholic and more susceptible to evaporation.

Staining Optimization Notes

1. Elastin Staining Notes: *This solution has a high alcohol content and is susceptible to evaporation. If staining on slides laying horizontally, monitor and add more stain as needed to prevent stain from drying on slide. Covering slide to reduce exposure to air currents may also help prevent evaporation. More differentiation may be needed if solution dries on tissue.*

2. Differentiation Notes: *Each slide may require a unique number of dips for optimal differentiation based on Elastin staining and tissue block used. Macroscopically, even well-differentiated slides will be greyish after this step (depending on amount of elastin and nuclei in tissue). Slides may also be checked microscopically during this step for optimal differentiation. When entire kit is complete, if elastin fibers are expected but not stained (over-differentiated), decrease number of dips on future slides. If fine elastin is seen but other tissue elements also remain greyish (under-differentiated), more dips will be required to remove excess stain on future slides. We suggest attempting under-differentiating with new tissues to locate all available elastin, and then increasing differentiation with subsequent slides until greyish background is no longer seen but fine elastin fibers remain.*

3. Muscle and Collagen Staining Notes (Steps 10-15): *Differentiation and staining intensity of the yellow and red colors may be controlled by increasing and/or decreasing staining times of the Biebrich Scarlet - Acid Fuchsin Solution (step 12), Phosphotungstic Acid Solution 5% (step 14), and Yellow Staining Solution (step 17). Always rinse off Yellow Stain Solution with absolute alcohol. Rinsing in water will very quickly remove stain and may alter results. Microscopically, some red or pink may remain in collagen after differentiation. This is acceptable and will still be displaced by the subsequent yellow stain.*

Procedure:

1. Deparaffinize sections if necessary and hydrate to distilled water.
2. Stain tissue section with Working Elastic Solution for 20 minutes.
See Elastin Staining Notes above
3. Rinse in running tap water for 1 minute followed by a rinse with deionized water.
4. Differentiate slide in Ferric Chloride (2%) Differentiating Solution by pouring into a small staining jar and dipping slide 10-20 times. **See Differentiation Notes above.** Continue differentiating if needed.
5. Rinse in tap water followed by a rinse in distilled water.
6. Apply Sodium Thiosulfate Solution (5%) and incubate for 1 minute.
7. Rinse well with distilled water.
8. Stain tissue with Alcian Blue Solution, pH 2.5 for 15-30 minutes.
9. Rinse slide with distilled water.
10. Stain slide in Biebrich Scarlet – Acid Fuchsin Solution for 2 minutes.
See Muscle and Collagen Staining Notes Above.
11. Rinse slide with distilled water.
12. Differentiate by applying two rounds of Phosphotungstic Acid Solution (5%) for 7 minutes each.
13. Rinse slide with distilled water.
14. Apply Acetic Acid Solution (1%) for 3 minutes.
15. Pour off Acetic Acid Solution (1%) and stain with Yellow Stain Solution for 10-20 minutes.
16. Rinse slide thoroughly in absolute alcohol. **Do not rinse with water, rinsing with water will remove yellow stain.**

17. Dehydrate in absolute alcohol, **do not dehydrate through graded alcohols containing water. Yellow stain may turn dehydrating alcohol yellow.**

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

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ScyTek Laboratories, Inc.
205 South 600 West
Logan, UT 84321
435-755-9848
U.S.A.



Emergo Europe
Prinsessegracht 20
2514 AP The Hague, The Netherlands