

TOULOUSE-LAUTREC (1864-1901)

French Artist recognized for his Posters and Lithographs of Parisian Nightlife

"TOULOUSE-LAUTREC 1-STEP TRICHROME Stain Kit" (Modified Gomori's)

CATALOG #HTGT-KIT

INTENDED USE:

BIOCARE's TOULOUSE-LAUTREC 1-STEP TRICHROME Stain kit is intended to identify an increase in collagenous connective tissue fibers or to differentiate between collagen and smooth muscle fibers.

PRINCIPLE OF TEST:

In the TOULOUSE-LAUTREC 1-STEP TRICHROME staining procedure, a plasma stain (chromotrope 2R) and a connective tissue fiber stain (aniline blue) are combined in a solution of phosphotungstic acid to which glacial acetic acid has been added. Phosphotungstic acid favors the red staining of muscle and cytoplasm. The tungstic ion is specifically taken up by collagen, and the connective tissue fiber stain is subsequently bound to this complex, coloring the collagen blue.

COMMENTS AND PRECAUTIONS:

BIOCARE MEDICAL uses only stains, which are certified by the **BIOLOGICAL STAIN COMMISSION**.

NOTE: Treatment with hot Bouin's solution is necessary to obtain satisfactory staining of muscle and collagen. Without this pretreatment color, differentiation of muscle and collagen will be very poor. Omit step if tissues were fixed in Bouin's solution initially. **DO NOT USE ANY REPLACEMENT BOUIN'S SOLUTION, SUCH AS SATURATED PICRIC ACID.** Tissue will not stain properly. Side by side research has been done using saturated picric acid, Bouin's 2000, a Bouin's substitute and Bouin's solution.

Rinsing in acetic acid after staining renders the shades of color more delicate and transparent.

SPECIMEN REQUIREMENTS:

Any well-fixed paraffin section cut 3-6 microns.

SOLUTIONS IN KIT: COMPONENTS AVAILABLE IN LARGER SIZES

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| <p>1. Bouin's Solution: CAT #HTBS-L (100 ml)
Saturated aqueous Picric acid
37% formalin
Acetic acid, glacial</p> <p>2. Weigerts Iron Hematoxylin, Solution "A"
CAT #HTWHS-A-L (100 ml)
Hematoxylin C.I. #75290
95% alcohol
Stabilizer</p> <p>3. Weigerts Iron Hematoxylin, Solution "B"
CAT #HTWHS-B-L (100 ml)
Ferric chloride
Hydrochloric acid
Distilled water</p> <p><i>Working Weigerts Iron Hematoxylin Solution:
Mix equal parts of Solution A and B.</i></p> | <p>4. TOULOUSE-LAUTREC 1-STEP TRICHROME Stain Solution: CAT #HTGTS-L (100 ml)
Chromotrope 2R C.I. #16570
Aniline Blue C.I. #42755
Glacial Acetic Acid
Phosphotungstic Acid
Distilled Water</p> <p>5. TL Acetic Acid Solution:
CAT #HTAAS-L2J (250 ml)
Acetic acid
Distilled water
Color indicator</p> <p>6. Acid Alcohol Solution:
CAT #HTAALCS-L2J (250 ml)
Hydrochloric Acid
Ethyl Alcohol
Distilled water</p> |
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"TOULOUSE-LAUTREC 1-STEP TRICHROME Stain Kit"

STORAGE AND STABILITY:

Store Weigerts Hematoxylin A & B Solution at room temperature (18-26°C) **in a dark place**. Stable for 12 months. **Working Weigerts Hematoxylin is stable for 1 week if capped and in a dark place.**
Store TOULOUSE-LAUTREC 1-STEP TRICHROME stain at room temperature (18-26°C). Stable for 12 months.

LIMITATIONS and WARRANTY:

There are no warranties, expressed or implied, which extend beyond this description of the product. BIOCARE is not liable for damages of any kind including personal injury or economic loss caused by this product.

QUALITY ASSURANCE AND CORRECTIVE ACTION GUIDELINES:

Cut sections between 3-5 microns. Practically every tissue has an internal control, so no other sections are needed, however, if a control is desired, uterus, small intestine, appendix, or fallopian tube will provide good material.

STANDARD STAINING METHOD:

1. Deparaffinize and hydrate to distilled water.
2. In a fume hood or well ventilated area, mordant in **preheated Bouin's solution at 62° to 64°C for 1 hour** (or at room temperature overnight). **Cool for 5-10 minutes.**

Note: We have found that the elevated temperature of **62° to 64°C for 1 hour in a water bath or Decloaking chamber** increases staining intensity of muscle tissue. If temperature reaches temperature higher than **68°C.**, tissue damage occurs.

Alternate microwave method:

Place slides in **40 ml** of Bouin's solution contained in a plastic coplin jar. Loosely cover coplin jar with lid before placing in microwave. **Microwave on 600 watts for 30 seconds.** Gently mix solution with beral pipet or applicator stick and **check with thermometer** to see if solution has reached **62° to 64°C.** (**Every microwave is different and should be checked for correct time and power setting**) incubate slides in heated Bouin's solution for **15 minutes** in a fume hood or well-ventilated area.

Note: We have found that multiple slides in the microwave give inconsistent staining results in muscle tissue. Preferred method of choice is the water bath method, using Biocare's **Decloaking chamber at 62° to 64°C for 1 hour**

3. Wash slides in tap water until sections are clear.
4. Stain in working Weigert's hematoxylin **5 minutes.**
5. Wash slides thoroughly in tap water.
6. Place in 0.5% Hydrochloric acid alcohol for **5 seconds.** (**Note this is a crucial step, to adjust to proper pH.**)
7. Wash in running tap water for **30 seconds** and rinse in two changes of distilled water.
8. Stain in TOULOUSE-LAUTREC 1-STEP TRICHROME Solution for **15 minutes.**
9. Wash slides in tap water.
10. Rinse in 0.5% Acetic acid **10 seconds.**
11. Rinse in distilled water.
12. Dehydrate through graded alcohols.
13. Clear in several changes of clearing agent.
14. Mount with resinous mounting media.

RESULTS:

Nuclei	black
Muscle fibers	red
Collagen	blue

REFERENCES:

Elbadawi, A.: Hexachrome modification of Movat's stain. Stain Technol. 51:249-253, 1976.
Gomori G.L. A rapid one-step trichrome. Amer J Clin Path. 1950; 20:661
Manual of Histologic Staining Methods of AFIP, 3rd ed, McGraw-Hill., 1968, pg 93
Histotechnology A Self-Instructional Text, 2nd Ed. F. L. Carson, pp.136, 1996
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