U.S. Corporate Headquarters
400 Valley Rd.
Warrington, PA 18976
1(800) 523-2575 / (215) 343-6484
1(800)343-3291 fax
info@polysciences.com

Polysciences Europe GmbH
Handelsstrasse 3
D-69214 Eppelheim, Germany
+(49) 6221-765767
+(49) 6221-764620 fax
info@polysciences.de

Polysciences Asia-Pacific, Inc.
2F-1, 207 Dunhua N. Rd.
Taipei, Taiwan 10595
(886) 2 8712 0600
(886) 2 8712 2677 fax
info@polysciences.tw

Gomori's Trichrome Stain Kit

INTRODUCTION

Gomori's One Step Trichrome refers to the multiple stain reaction of this reagent only. However, adequate staining of all cell components requires several other solutions to obtain balance in the tissue. The mordant with Bouin's Fixative is used to drop the pH and for protein interaction in the section. While this mechanism is not clear the stain works best with this step included. The use Wiegert's Iron Hematoxylin for nuclear detail is the choice for this stain. The acid step to differentiate at the end is critical for showing fine muscle fibers. The usual acid is Acetic Acid and does produce a good differentiation of the fibers; the use of Hydrochloric Acid is better and defines the fibers more clearly. The choice of acid is for the laboratory to decide based on the requirements of the investigator or pathologist.

FIXATION

10% Neutral Buffered Formalin or alcohol can be used. Sections should be cut at 4µ to 5µ.

DEPARAFFINIZE

Slides should be deparaffinized through xylene or substitute to remove the paraffin from the section and descending grades of alcohol to distilled water just prior to staining.

STAINING PROCEDURE: ROUTINE PROCEDURE FOR ROOM TEMPERATURE

1. Bouin's Fixative for 1 hour at 56°C or overnight at room temperature. The dish must be covered at all times during the mor- dant. Allow the solution to cool for 5 to 10 minutes after removal from the oven.
2. Wash well in running tap water to remove all of the yellow colour from the section.
3. Stain with Wiegert's Iron Hematoxylin. Mix equal amounts of Solution A and Solution B just prior to staining. Stain for 10 minutes. This solution can be used for several days.
4. Wash well in running tap water for 10 minutes to remove excess stain
5. Stain with Gomori's Trichrome Stain for 15 to 20 minutes. Solution can be filtered and reused.
6. Place in 0.5% Acetic Acid ***OR*** 0.5% Hydrochloric Acid for 2 minutes as a rinse and to differentiate the stain. The stain can be decolorized by using 1% Acetic Acid Aqueous with 0.7% Phosphotungstic Acid Solution Aqueous, if overstaining occurs.
7. Dehydrate up through graded ethyl alcohols to absolute ethyl alcohol and into xylene or substitute. Coverslip with Poly-Mount or other media.

STAINING PROCEDURE: HEATED MICROWAVE PROCEDURE

Steps 2 to 7 must be done under a hood and the microwave must be vented or, under the hood. Larger amounts of stain can be made and heated by extending the times as indicated in the procedure. Heating in a Coplin jar will require 15 to 30 seconds to reach 60°C and 1 to 1 1/2 minutes for 250 mL to reach 60°C in a larger staining dish on HIGH in a microwave or as directed by the manufacturer using a probe. Please calibrate your oven by checking this temperature with room temperature distilled water for accurate control.

Steps 1-7 on next page.

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1. Deparaffinize as above.
2. Heat Bouin's Solution in the microwave at high power for 15 seconds in a plastic Coplin jar, loosely capped. Remove and place the slides in the solution for 15 minutes, at room temperature under the hood.
3. Wash in running tap water to remove the yellow colour.
4. Stain Wiegert's Iron Hematoxylin mixed in equal parts of Solutions A & B. Heat the Working Solution for 15 seconds in the microwave on high in a plastic Coplin jar. Remove from the microwave and place slides in the stain. Stain for 2 minutes.
5. Wash in running tap water for 3-5 minutes.
6. Heat Gomori's Trichrome Solution for 15 to 30 seconds to the temperature of 60°C in a plastic Coplin jar, loosely capped. Remove the slides from water and place in the stain for 8 to 12 minutes. The temperature decrease lose over this time will not effect the staining intensity and will assist in holding the tissue on the slides.
7. Rinse in either 0.5% Acetic Acid ***OR*** 0.5% Hydrochloric Acid Solution for 2 minutes. The solution can be heated for 10 seconds in a plastic Coplin jar to shorten the time to 30 seconds to 1 minute, if desired. The stain can be differentiated by using 1% Acetic Acid Aqueous with 0.7% Phosphotungstic Acid Solution Aqueous, if overstaining occurs. Dehydrate through graded ascending ethyl alcohols to xylene and coverslip with Poly-Mount or other media.

Results:	Nuclei	Black
	Cytoplasm, Keratin, muscle fibers	Red
	Collagen and mucus	Green or Blue

ORDERING INFORMATION

Cat. #	Description	Size
24205	Each Kit contains one each of the following:	
	Bouins Fixative	250mL
	Wiegert's Iron Hematoxylin Solutions	
	Solution A	250mL
	Solution B	250mL
	Gomori's One Step Trichrome	250mL
	0.5% Glacial Acedic Acid Aq	250mL
	0.5% Hydrochloric Acid Aq	250mL
08381	Poly-Mount	120mL
		940mL

TO ORDER

In The U.S. Call: 1(800) 523-2575 • (215) 343-6484
In The U.S. Fax: 1(800) 343-3291 • (215) 343-0214

In Germany Call: +(49) 6221-765767
In Germany Fax: +(49) 6221-764620

In Asia Call: (886) 2 8712 0600
In Asia Fax: (886) 2 8712 2677

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