

CHROMOTROPE 2R / FAST GREEN F.C.F. REAGENT

IVD *In vitro* diagnostic medical device



Green counterstain used with Gomori Trichrome kit

INSTRUCTIONS FOR USE

REF Catalogue number: CFG-OT-100 (100 mL)

Introduction

Chromotrope 2R / Fast Green F.C.F. reagent is a component of Gomori Trichrome kit used for analysis of collagen fibers in liver and kidneys. This kit is usually used in order to achieve easier differentiation of collagen and smooth muscle fibers, as well as for distinguishing destroyed fibers (present in cases of mitochondrial myopathies).

Product description

- **CHROMOTROPE 2R / FAST GREEN F.C.F. REAGENT** – Reagent containing Chromotrope 2R and Fast Green F.C.F dyes with stabilizers.

Example of staining with Chromotrope 2R / Fast Green F.C.F. reagent as a component of Gomori Trichrome kit

Other sections and reagents necessary for staining:

- Fixatives such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydrating/rehydrating agent, such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95 and Histanol 100
- Clearing agents, such as BioClear xylene or a substitute, such as BioClear New agent on the aliphatic hydrocarbons basis
- Infiltration and fitting agent, such as BioGnost's granulated paraffin BioWax Plus, BioWax 52/54, BioWax 56/68, BioWax Blue, BioWax Micro.
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount DPX Low Eco, BioMount C, BioMount Aqua, Canada Balsam
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE, VitroGnost COLOR or one of more than 30 models of BioGnost's VitroGnost glass slides
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade
- BioGnost's reagents that comprise Gomori Trichrome kit: Bouin's solution (product code BOU-OT-100), Hematoxylin, Weigert A (product code HEMA-OT-100), Ferri reagent, Weigert B (product code FR-OT-100) and Differentiating reagent for Gomori Trichrome kit (product code RDG-OT-100)

Preparing the histological sections for staining

- Fixate the sample (Formaldehyde NB 4%, Formaldehyde NB 10%), rinse with water and dehydrate through series of ascending alcohol solutions (Histanol 70, Histanol 80, Histanol 95 and Histanol 100).
- Clear the sample with intermedium; in xylene (BioClear) or in a xylene substitute (BioClear New).
- Infiltrate and fit the sample in paraffin (BioWax Plus, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to 4-6 μm slices and place them on a VitroGnost glass slide.

Sample staining procedure

Pour the reagents into glass staining jars (Coplín, Hellendahl or Schifferdecker), return to original bottles after staining. Close tightly. Filter the reagents if necessary.

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate using 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate using 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled (demi) water	2 min
5.	Immerse into Bouin's solution	60 min at 56°C or over night at room temperature
6.	Cool the section down at room temperature	10 min
7.	Rinse under tap water	10 seconds
8.	Rinse in distilled water	10 seconds
9.	Prepare Weigert hematoxylin working solution: mix equal volumes of Hematoxylin, Weigert A and Ferri reagent, Weigert B	
	Note: working solution is stable for approximately 2 weeks. Prepare the working solution of volume adequate for staining test sections	
10.	Immerse into Weigert hematoxylin working solution and let it react	5 min
11.	Rinse under tap water	5 min
12.	Immerse in Chromotrope 2R / Fast Green F.C.F. reagent	5-10 minutes
13.	Rinse in distilled water	5 dips
14.	Immerse in Differentiating reagent for Gomori Trichrome kit	2 min
15.	Rinse in distilled water	5 dips
16.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
17.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
18.	Dehydrate using 100% alcohol (Histanol 100)	2 min
19.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing apply an appropriate BioMount medium for covering/mounting on the section. If BioClear xylene was used, use one of BioGnost's mounting xylene-based media (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate covering agent is BioMount New. Cover the section with a VitroGnost cover glass.

Result

Collagen - green
 Muscle tissue - pink
 Nuclei – blue-purple
 Erythrocytes - red to orange

Note

Staining procedures are not standardized and they depend on standard operating procedures of individual laboratories and the experience of the personnel conducting the staining procedure. Intensity of staining depends on the period of immersion in the dye. Depending on personal requests and standard laboratory operating procedures, sample processing and staining can be carried out according to other protocols.

Preparing the sample and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples with modern technology and mark them clearly. Follow the manufacturer's instructions for handling. In order to avoid mistakes, the staining procedure and diagnostics should only be conducted by authorized and qualified personnel. Use only microscope according to standards of the medical diagnostic laboratory.

Safety at work and environmental protection

Handle the product in accordance with safety at work and environmental protection guidelines. Used solutions and out of date solutions should be disposed of as special waste in accordance with national guidelines. Chemicals used in this procedure could pose danger to human health. Tested tissue specimens are potentially infectious. Necessary safety measures for protecting human health should be taken in accordance with good laboratory practice. Act in accordance with signs and warnings notices printed on the product's label, as well as in BioGnost's material safety data sheet.
















Storing, stability and expiry date

Keep Chromotrope 2R / Fast Green F.C.F. reagent in a tightly sealed original packaging at temperature of +15 to +25°C. Keep in dry places, do not freeze and avoid exposing to direct sunlight. Date of manufacture and expiry date are printed on the product's label.

References

1. Bancroft, J.D., Gamble, M. (2002), Theory and practice of Histological Techniques, Churchill Livingstone, New York.
2. Prophet, Mills, Arrington, Sobin (1968), Laboratory methods in histotechnology. Stain methods of the Arm Forces Institute of Pathology, Washington D.C.
3. Carson, F. L. (1997), Histotechnology: a self-instructional text. American Society for Clinical Pathology (2nd edition), Singapore.

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	Refer to the supplied documentation		Storage temperature range		Number of tests in package		Product code		European Conformity	 BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com	
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until		Lot number		Manufacturer		
	For <i>in vitro</i> diagnostic use only		Keep in dry place		Caution - fragile						