MARTIUS YELLOW, powder dye, C.I. 10315

IVD In vitro diagnostic medical device

Manchester Yellow, Golden Yellow, Martinsgelb, Acid Yellow 24 For staining erythrocytes yellow in trichrome staining methods

INSTRUCTIONS FOR USE

REF Product code: MAY-P-25 (25 g)

Introduction

Histology, cytology and other related scientific disciplines study the microscopic anatomy of tissues and cells. In order to achieve a good tissue and cellular structure, the samples need to be stained in a correct manner. Martius Yellow dye is used for cytoplasmic staining with several trichrome methods used in human and animal histology, such as MSB (<u>Martius Scarlet Blue</u>) method for staining fibrin (acc. to Lendrum, 1962) that is often used in forensic pathology to assess mode of injury. Martius Yellow dye is also used as counterstain for PAS (<u>periodic acid-Schiff</u>) stain for demonstrating parasitic amoebae.

Product description

• MARTIUS YELLOW - powder dye for preparing solutions for cytoplasmic staining.

Example of using Martius Yellow as a component of MSB staining method

Other preparations and reagents used for perapring dye solutions and with staining process

- · Concentrated (glacial) acetic acid, phosphotungstic acid
- Powder dyes: Brilliant Crystal Scarlet and Methyl Blue
- 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 52/54, BioWax Plus 56/58, BioWax 56/68, BioWax Blue, BioWax Micro
- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE or one of more than 30 models of BioGnost's glass slides
- Covering agents for microscopic sections and mounting cover glass, such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C, BioMount Aqua
- VitroGnost cover glass, dimensions range from 18x18mm to 24x60mm
- · Reagent for acid-fast nuclear staining, such as BioGnost's Weigert hematoxylin (Hematoxylin W kit)

Preparing dyes solution:

Martius Yellow, solution

- Dissolve 0.5 g of Martius Yellow powder dye in 100 mL of 95% alcoholic solution (Histanol 95).
- Add 2 g of phosphotungstic acid, mix well.

Brilliant Crystal Scarlet solution

- Dissolve 1 g of Brilliant Crystal Scarlet powder dye in 100 mL of distilled (demi) water.
- Add 2 mL of glacial acetic acid and mix well.
- Phosphotungstic acid solution
- Dissolve 1 g of phosphotungstic acid in 100 mL of distilled (demi) water.

Methyl Blue, solution

- Dissolve 0.5 g of Methyl Blue powder dye in 100 mL of distilled (demi) water.
- Add 1 mL of glacial acetic acid and mix well.
- Acetic acid solution
- Add 1 mL of glacial acetic acid to 100 mL of distilled (demi) water. Mix well.

Preparing histological sections for staining

- Fix the tissue sample tightly (4% NB Formaldehyde, 10% NB Formaldehyde), 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 52/54, BioWax Plus 56/58, BioWax 56/58, BioWax Blue, BioWax Micro).
- Cut the paraffin block to 4-6 μ m slices and place them on a VitroGnost glass slide.

NOTE

Apply the reagent so it completely covers the section.

Staining procedure

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.	Stain using Weigert hematoxylin	10 min					
6.	Rinse the section quickly under tap water						
7.	Rinse using 95% alcohol (Histanol 95)						
8.	Stain with Martius Yellow solution	2 min					
9.	Rinse in distilled (demi) water						
10.	Stain using Brilliant Crystal Scarlet solution	10 min					

13.	Rinse in distilled (demi) water						
14.	Treat with phosphotungstic acid solution	until the excessive red dye in collagen is washed off					
15.	Rinse in distilled (demi) water	2 min					
16.	Stain using Methyl Blue solution	5 minutes or until collagen is sufficiently stained					
17.	Rinse in acetic acid solution						
18.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 30 seconds each					
19.	Dehydrate using 100% alcohol (Histanol 100)	2 exchanges, 1 min each					
20.	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 VitroGnost cover glass.

Results

Nuclei - blue Muscles - red Fibrins - red (young clusters may be stained yellow, and older ones blue) Collagen - blue Erythrocytes - yellow

Note

The mentioned formulation is only one of the ways of preparing the dye solution. Depending on personal requests and standard laboratory operating procedures, the dye solution can be prepared 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. In order to avoid an erroneous result, a positive and negative check is advised before application.

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 Martius Yellow powder dye in a tightly sealed original packaging at temperature between $+15^{\circ}$ C and $+25^{\circ}$ C. Keep in dry places, do not freeze and avoid exposure to direct sunlight. Expiry date is stated on the product's label.

References

- 1. Bancroft, J.D. and Gamble, M. (2008) Theory and Practice of Histological Techniques. 6th Edition, Churchill Livingstone, Elsevier, China
- 2. Conn, J. (1977): Biological Stains, 9th ed. Baltimore: Williams and Wilkins Co.
- 3. Lillie, R.D. Conn's Biological Stains, Williams & Wilkins, Baltimore, MD., U.S.A.
- 4. Gurr, E., (1971): Synthetic dyes in biology, medicine and chemistry, Academic Press, London, England.

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Â	Refer to the supplied documentation	°C-	Storage temperature range	Σ	Number of tests in package	REF	Product code	C	E	European Conformity	BIOGNOST Ltd. Medjugorska 59 10040 Zagreb	C	E
[]i	Refer to supplied instructions	歉	Keep away from heat and sunlight		Valid until	LOT	Lot number		•	Manufacturer	CROATIA www.biognost.com		
IVD	For <i>in vitro</i> diagnostic use only	1	Keep in dry place	4	Caution - fragile								