

ORCEIN, C.I. (ed.1) 1242

IVD *In vitro* diagnostic medical device



Natural Red 28, Orcein Synthetic

For staining elastic tissues and antigen of the hepatitis B virus

INSTRUCTIONS FOR USE

REF Catalogue number: ORC-P-5 (5 g)

ORC-P-10 (10 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. Orcein dye is used for staining elastic fibers and lamellas. It can also stain plant chromosomes, human sex chromatin, and is used as stain for cellular nuclei. One of its prominent uses is also demonstrating liver inclusions, especially for hepatitis B antigen, but also for clusters of proteins that contain copper.

Product description

- **ORCEIN** - Biological Stain Commission (BSC) certified powder dye for preparing dye solution

Other sections and reagents that may be used in 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
- Potassium permanganate
- Oxalic acid
- Sulfuric acid
- Periodic acid, 5% solution (product code PK05-OT-30, PK05-OT-100, PK05-OT-250, PK05-OT-500)
- Acid alcohol (product code KA-OT-1L, KA-OT-2L)
- Hydrochloric acid

Preparing the solution for staining procedure

Potassium permanganate solution:

- Mix 0.15 g of potassium permanganate and 100 ml of distilled (demi) water. Add 5 ml of 3% sulfuric acid.
- Mix well, discard after use.

5% oxalic acid solution

- Dissolve 5 g of oxalic acid in 100 ml of distilled (demi) water.
- Mix well. The solution is stable for 1 year

Orcein solution

- Dissolve 1 g of Orcein powder dye in 100 ml of 70% alcohol (Histanol 70). Add 1 ml of hydrochloric acid.
- Mix well. The pH value must be between 1 and 2. Let the solution set for at least 48 hours. Filter before use. Stable for 6 months.

Preparing the histological sections for staining

- Fix the sample (Formaldehyde NB 4%, Formaldehyde NB 10%, Bouin's solution), 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 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.

Histological sections 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.	Treat with potassium permanganate solution	10 min
6.	Rinse in distilled (demi) water	
7.	Treat with 5% oxalic acid until discoloration	
8.	Rinse in tap water	
9.	Rinse in distilled (demi) water	
10.	Treat with Periodic acid, 0.5% solution	5 min
11.	Rinse in tap water	
12.	Rinse in distilled (demi) water	
13.	Stain with orcein solution; standard staining at room temperature or	4-16 hours

	of heating in microwave oven with low temperature setting. Let it set for 30 minutes. Check the staining level under microscope. If the coloration is not strong enough, repeat the procedure	30-45 seconds
14.	Dehydrate using 95% alcohol (Histanol 95)	
15.	Rinse using 70% alcohol (Histanol 70)	
16.	Differentiate using Acid alcohol if necessary (check under microscope)	
17.	Rinse using 70% alcohol (Histanol 70)	
18.	Dehydrate using 95% alcohol (Histanol 95)	2 exchanges, 30 seconds each
19.	Dehydrate using 100% alcohol (Histanol 100)	2 exchanges, 1 min each
12.	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.

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. Time periods of staining processes are not entirely standardized and they approximately correspond to clinical and laboratory practical experience. Intensity of staining depends on the period of immersion in the dye. Real staining protocol depends on personal requests and priorities.

Results

Hepatitis B antigens, elastic fibers - dark brown to purple

Proteins containing copper - dark purple

Background - light brown to purple

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. Both positive and negative controls are recommended before applying.

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 which is available on demand.














Storing, stability and expiry date

Keep Orcein powder dye in a tightly sealed original packaging at temperature between 15°C and 25°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. Conn, J. (1977): Biological Stains, 9th ed. Baltimore: Williams and Wilkins Co.
2. Carson, F. L., Hladik, C. (2009): Histotechnology: A Self-Instructional Text, 3rd ed., Chicago: ASCP Press
3. Lillie, R.O. (1969): Biological Stains, 8th ed., Baltimore: Williams & Wilkins Co.
4. Luna, L. (1978): Recommended Procedure for Demonstrating Hepatic B Antigen in Paraffin Sections, Histo-Logic, Vol. VIII. No. 4, LAB-TEK Div., Miles Lab. Inc.

ORC-P-X, V2, 15 February 2017, AKVR

 Refer to the supplied documentation	 Storage temperature range	 Number of tests in package	 Product code	 European Conformity
 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		

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