

ORCEIN KIT

IVD *In vitro* diagnostic medical device



Kit for visualization of surface hepatitis B (HBsAg) antigens observable as viral inclusion bodies, elastic fibers and protein complexes with copper

INSTRUCTIONS FOR USE

REF Catalogue number: ORC-100T (for 100 tests)

ORC-K-250 (5x250 mL)

Introduction

Orcein kit is used for identification of inclusion bodies of surface hepatitis B (HBsAg) antigens, elastic fibers and protein complexes with copper. It may be used with the sections embedded in paraffin, but also with frozen sections. It is also recommended to fix the sections prior to procedure by using neutral buffered formaldehyde.

Product description

ORCEIN KIT - Five-reagent kit for visualization of surface hepatitis B (HBsAg) antigens, elastic fibers and protein complexes with copper

The kit contains:	100 tests (ORC-100T)	5 x 250 mL (ORC-K-250)
Orcein reagent	100 mL (ORCR-OT-100)	250 mL (ORCR-OT-250)
Potassium permanganate, 1% solution	30 mL (KP1-OT-30)	250 mL (KP1-OT-250)
Sulfuric acid, 0.3% solution	30 mL (SK03-OT-30)	250 mL (SK03-OT-250)
Oxalic acid, 2% solution	30 mL (OKS2-OT-30)	250 mL (OKS2-OT-250)
Acid alcohol, Orcein	100 mL (KAO-OT-100)	250 mL (KAO-OT-250)

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

Preparing the 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 µm slices and place them on a VitroGnost glass slide.

NOTE

Apply the reagent so it completely covers the section.

Sample staining procedure

a) using kit for 100 tests (ORC-100T)

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.	Apply 5 drops of Potassium permanganate, 1% solution and 5 drops of Sulfuric acid, 0.3% solution	let it set for 10 min
6.	Drain the section without rinsing and proceed to the next step	
7.	Treat with Oxalic acid, 2% solution (≥5 drops)	let it set for 10 min
8.	Rinse in distilled (demi) water	
9.	Immerse in Orcein reagent	let it set for 1-4 hours
	Note: Pour the reagent into glass staining jars (Coplin, Hellendahl or Schifferdecker); close the jar during staining in order to reduce the possibility of evaporation. Return to the original bottle after staining and close tightly.	
10.	Rinse using 70% alcohol (Histanol 70)	5 dips
11.	Immerse into Acid alcohol, Orcein and differentiate the section	1-10 dips
	Note: this step may be skipped or modified, depending on the needs of an individual laboratory	
12.	Dehydrate using 70% alcohol (Histanol 70)	10 exchanges, 1 second each
13.	Dehydrate using 95% alcohol (Histanol 95)	10 exchanges, 1 second each
14.	Dehydrate using 100% alcohol (Histanol 100)	10 exchanges, 1 second each
15.	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.

b) using five 250 ml reagent kit (ORC-K-250)

Pour the reagents into glass staining jars (Coplin, 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.	Mix equal volumes of Potassium permanganate, 1% solution and Sulfuric acid, 0.3% solution and immerse the section	let it set for 10 min
	Note: dispose of the solution after use	
6.	Drain the section and immerse it in Oxalic acid, 2% solution	let it set for 10 min
7.	Rinse in distilled (demi) water	
8.	Immerse in Orcein reagent	let it set for 1-4 hours
	Note: Cover the jar with reagent during staining in order to reduce the possibility of evaporation. Return to the original bottle after staining and close tightly.	
9.	Rinse using 70% alcohol (Histanol 70)	5 dips
10.	Immerse into Acid alcohol, Orcein and differentiate	1-10 dips
	Note: this step may be skipped or modified, depending on the needs of an individual laboratory	
11.	Dehydrate using 70% alcohol (Histanol 70)	10 exchanges, 1 second each
12.	Dehydrate using 95% alcohol (Histanol 95)	10 exchanges, 1 second each
13.	Dehydrate using 100% alcohol (Histanol 100)	10 exchanges, 1 second each
14.	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

Elastic fibers, HBsAg, protein complexes with copper - red-purple-brown

Note

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.

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 Orcein kit in a tightly sealed original packaging at temperature of 15 °C to 25°C. 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., Livingstone, C. Theory and practice of Histological Techniques – 5° edizione 2002.
2. Deadhar, K.P., Tapp, E., Scheuer, P.J. (1975): Orcein staining of Hepatitis B Antigen in paraffin section of Liver Biopsis, *Journal of Clinical Pathology*, vol. 28: pp. 66-70
3. Salaspuro, M., Sipponen, P. (1976): Demonstration of an intracellular copper-binding protein by Orcein staining in long-standing cholestatic liver diseases, Gut, volume: 17, p 787-790.

ORC-X, V7-EN4, 27 March 2019, IŠP/VR

	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 in vitro diagnostic use only		Keep in dry place		Caution - fragile				

 BIOGNOST Ltd.
Medjugorska 59
10040 Zagreb
CROATIA
www.biognost.com

