

ORCEIN KIT

CE IVD *In vitro* diagnostic medical device

Classified acc. to Regulation (EU) 2017/746 - Class A device

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

INSTRUCTION FOR USE



BASIC UDI number	385889212HPC30708STARVF	
EMDN code	W01030708	
REF Catalogue number	Volume	UDI-DI number
ORC-100T	100 tests	03858890004557
ORC-K-250	5x250 mL	03858890008357

Intended use and test principle

The Orcein kit is used for the identification of hepatitis B virus surface antigens (HBsAg) inclusion bodies, elastic fibers and protein complexes with copper. It can be used on samples embedded in paraffin, but also on frozen sections. It is preferable that the samples are pre-fixed with neutrally buffered formaldehyde.

Product description

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

The kit contains:	100 tests (ORC-100T)	5 x 250 mL (ORC-K-250)	Storage temperature
Orcein reagent	100 mL (ORCR-OT-100)	250 mL (ORCR-OT-250)	15-25 °C
Potassium permanganate, 1% solution	30 mL (KP1-OT-30)	250 mL (KP1-OT-250)	15-25 °C
Sulfuric acid, 0.3% solution	30 mL (SK03-OT-30)	250 mL (SK03-OT-250)	15-25 °C
Oxalic acid, 2% solution	30 mL (OKS2-OT-30)	250 mL (OKS2-OT-250)	15-25 °C
Acid alcohol, Orcein	100 mL (KAO-OT-100)	250 mL (KAO-OT-250)	15-25 °C

Additional reagents and materials that can be used in staining

- Fixative agents such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydration/rehydration agents such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95, and Histanol 100
- Clearing agents, such as BioClear xylene or BioClear New, an aliphatic hydrocarbon-based xylene substitute
- Infiltration and embedding agents such as BioGnost's granulated paraffins BioWax 52/54, BioWax 56/58, BioWax Plus 56/58, BioWax Blue
- Microscopic slide covering agents and cover glass mountants such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount DPX New, BioMount C, BioMount Aqua
- VitroGnost slides and coverslips for use in histopathology and cytology
- Immersion media such as BioGnost's Immersion Oil, Immersion Oils types A, C, FF, 37, or Immersion Oil Tropical Grade

Preparation of histological sections for staining

- Fix (Formaldehyde NB 4%, Formaldehyde NB 10%) and process the tissue sample
- Embed the tissue in a paraffin block (BioWax 52/54, BioWax 56/58, BioWax Plus 56/58, BioWax Blue)
- Cut the paraffin block into 4-6 micron thin slices and mount on a VitroGnost microscope slide

NOTE

Apply the reagent to completely cover the section.

Sample staining procedure

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

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 changes, 2 minutes each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 changes, lasting 5 and 3 minutes
3.	Rehydrate in 95% alcohol (Histanol 95)	2 minutes
4.	Rehydrate in distilled/demineralized water	2 minutes
5.	Apply 5 drops of Potassium permanganate, 1% solution and 5 drops of Sulfuric acid, 0.3% solution	leave it on for 10 minutes
6.	Drain the section and proceed to the next step without rinsing	
7.	Treat with Oxalic acid, 2% solution (apply \geq 5 drops)	leave it on for 10 minutes
8.	Rinse in distilled/demineralized water	
9.	Immerse in Orcein reagent	leave to act for 1-4 hours
	Note: Pour the reagent into the staining containers (Coplin, Hellendahl or Schifferdecker type) and cover during staining to reduce the possibility of evaporation; after staining return to the original bottle and close tightly.	
10.	Rinse in 70% alcohol (Histanol 70)	5 dips
11.	Immerse in Acid alcohol, Orcein and differentiate the section	1-10 dips
	Note: this step can be skipped or modified depending on the requirements of the individual laboratory	
12.	Dehydrate in 70% alcohol (Histanol 70)	10 dips for 1 second
13.	Dehydrate in 95% alcohol (Histanol 95)	10 dips for 1 second
14.	Dehydrate in 100% alcohol (Histanol 100)	10 dips for 1 second
15.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 minutes each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium. If BioClear xylene was used, use one of BioGnost's xylene-based mountants (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate mountant 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 (type Coplin, Hellendahl or Schifferdecker) and return them to the original bottles after staining. Close well. If necessary, filter the reagents.

A mixture of Potassium permanganate and Sulfuric acid solutions in step no. 5 is intended for single use. Prepare the volume of solution that will be used in the staining process, and throw it away after use.

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 changes, 2 minutes each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 changes, lasting 5 and 3 minutes
3.	Rehydrate in 95% alcohol (Histanol 95)	2 minutes
4.	Rehydrate in distilled/demineralized water	2 minutes
5.	Mix an equal volume of Potassium permanganate, 1% solution and Sulfuric acid, 0.3% solution and immerse the section	leave it on for 10 minutes
6.	Drain the section and immerse it in Oxalic acid, 2% solution	leave it on for 10 minutes
7.	Rinse in distilled/demineralized water	
8.	Immerse in Orcein reagent	leave to act for 1-4 hours
	Note: Cover the container with the reagent during staining to reduce the possibility of evaporation; after dyeing, return to the original bottle and close tightly	
9.	Rinse in 70% alcohol (Histanol 70)	5 dips
10.	Immerse in Acid alcohol, Orcein and differentiate the section	1-10 dips
	Note: this step can be skipped or modified depending on the requirements of the individual laboratory	
11.	Dehydrate in 70% alcohol (Histanol 70)	10 dips for 1 second
12.	Dehydrate in 95% alcohol (Histanol 95)	10 dips for 1 second
13.	Dehydrate in 100% alcohol (Histanol 100)	10 dips for 1 second
14.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 minutes each

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

Result

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

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in this Instruction for use may cause differences in staining results.

Sample preparation and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples using modern technology and mark them clearly. Be sure to follow the manufacturer's handling instructions. To avoid errors, staining, mounting of the slides, and diagnosis can only be carried out by qualified personnel. Use a microscope equipped according to medical diagnostic laboratory standards. To avoid a false result, it is recommended to use a positive and negative control.

If a serious incident occurs during use of this product or as a result of its use, please report it to the manufacturer or authorized representative and competent authority.

Safety at work and environmental protection

Handle the product in accordance with occupational health and environmental protection guidelines. Used and expired solutions must be disposed of as special waste following national guidelines. Reagents used in this procedure can pose a danger to human health. The examined tissue samples are potentially infectious, and it is necessary to take the measures needed to protect human health in accordance with the guidelines of good laboratory practice. It is mandatory to read and act according to the information and warning signs printed on the product label and in the Safety Data Sheet, which is available on request.

Storage, transport, stability, and shelf life

Upon receipt, store the product in a dry place and well-closed original packaging at a temperature of +15 °C to +25 °C. Do not freeze or expose to direct sunlight. After first opening, the product can be used until the specified expiry date, if stored properly. The production date and expiration date are printed on the product label.

Literature

1. Bancroft, J.D., Gamble, M. Livingstone, C. *Theory and practice of Histological Techniques* – 5° edizione 2002.
2. Deadhar, KP, Tapp, E., Scheuer, PJ (1975): Orcein staining of Hepatitis B Antigen in paraffin section of Liver Biopsis, *Journal of Clinical Pathology*, vol. 28: p. 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.

Warnings and precautions regarding the materials contained in the product:	
	<p>H225 Highly flammable liquid and vapour.</p> <p>P210 Keep away from heat, hot surfaces, sparks, open flames and other sources of ignition. No smoking.</p> <p>P233 Keep container tightly closed.</p> <p>P280 Wear protective gloves/protective clothing/eye protection/face protection.</p>

ORC-IFU_EN6, 31 July, 2024 LO/IŠP

 Manufacturer	 Batch code	 Temperature limit	 <i>In vitro</i> diagnostic medical device	 Unique device identifier
 Date of manufacture	 Catalogue number	 Consult instructions for use	 Contains sufficient for <n> tests	
 Use-by date	 Fragile, handle with care	 Caution	 European conformity	

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Version	Description / reason for change	Date
6.	Revised in acc. to Regulation (EU) 2017/746 - IVDR	31.07.2024.