

# LADEWIG'S REAGENT

IVD *In vitro* diagnostic medical device



## For use with Ladewig Trichrome kit

### INSTRUCTIONS FOR USE

REF Product code: LDWR-OT-100

#### Introduction

Ladewig reagent is a component of Ladewig Trichrome kit used for staining histology samples with emphasis on differential counterstaining of muscle and collagen tissues. Compared to other BioGnost's Trichrome kits (Masson and Mallory), the staining procedure is shorter and simpler because of a single incubation in the Ladewig reagent that stains all the necessary structures simultaneously. Ladewig reagent contains Aniline Blue that binds to collagen providing a characteristic blue staining, Acid Fuchsin that dyes the contrast structures red, and Orange G that stains erythrocytes.

#### Product description

- **LADEWIG REAGENT** – powder dye aqueous solution with added oxalic acid

#### Example of Ladewig reagent use with Ladewig Trichrome kit

#### 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 52/54, BioWax Plus, BioWax 56/68, BioWax Blue.
- Covering agent 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
- 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
- Other components of Ladewig Trichrome kit: Hematoxylin, Weigert A (HEMA-OT-100), Ferri reagent, Weigert B (FR-OT-100) and Phosphotungstic acid, 1% solution (FVK1-OT-100)

#### Preparing the histological sections for staining

- Fix 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 section in paraffin (BioWax 52/54, BioWax Plus, BioWax 56/58, BioWax Blue).
- Cut the paraffin block to **4-6**  $\mu\text{m}$  slices and place them on a VitroGnost glass slide.

#### NOTE

Apply the reagent so it completely covers the section.

#### Sample staining procedure

##### Using four-reagent 100 mL kit (LDW-K-100)

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.	Prepare the 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	
6.	Immerse into Weigert hematoxylin working solution and let it react	3-5 minutes
7.	Rinse under tap water	1 min
8.	Immerse into Phosphotungstic acid, 1% solution	3 min
9.	Rinse shortly in distilled water	
10.	Immerse in Ladewig reagent	4 min
11.	Rinse in distilled water	until the excessive dye is washed off of the section
12.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
13.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
14.	Dehydrate using 100% alcohol (Histanol 100)	2 min
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 VitroGnost cover glass.

## Result

Cytoplasm - red  
Nuclei - brown to black  
Muscle fibers - brown to red  
Collagen - blue to purple  
Erythrocytes - 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 taken care of as a 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 Ladewig reagent in a tightly sealed original packaging at temperature of +15 °C 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. Gray, Peter. (1954): The Microtomist's Formulary and Guide.

LDWR-OT-100, V1, 24 March 2023, KB/IŠP

	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](http://www.biognost.com)

