BIOGNOST®

LADEWIG'S TRICHROME KIT

IVD *In vitro* diagnostic medical device CE

Four-reagent muscle and collagen fiber staining kit

INSTRUCTIONS FOR USE

REF Product code: LDW-100T (for 100 tests) LDW-K-100 (4 x 100 mL)

Introduction

Ladewig's Trichrome kit is used for staining histology sections, with emphasis on muscle and collagen tissue differential counterstaining. The staining procedure is shorter and simpler compared to other BioGnost's Trichrome kits (Masson and Mallory) because during incubation performed only with the Ladewig's reagent all the target structures are being stained at the same time. Ladewig's reagent contains Aniline blue dye that binds to collagen enabling characteristic blue coloration; Acid Fuchsin stains contrasting structures red, and Orange G stains erythrocytes.

Product description

- **LADEWIG'S TRICHROME KIT** - Four-reagent muscle and collagen fiber staining kit

The kit contains:	100 tests (LDW-100T)	4 x 100 mL (LDW-K-100)
Hematoxylin, Weigert A	30 ml (HEMA-OT-30)	100 ml (HEMA-OT-100)
Ferri reagent, Weigert B	30 ml (FR-OT-30)	100 ml (FR-OT-100)
Phosphotungstic acid, 1% solution	30 mL (FVK1-OT-30)	100 mL (FVK1-OT-100)
Ladewig's reagent	30 mL (LDWR-OT-30)	100 mL (LDWR-OT-100)

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 56/68, BioWax Blue
- 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 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 sample in paraffin (BioWax Plus, BioWax 56/58, BioWax Blue).
- 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 (LDW-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 Hematoxylin, Weigert A and 5 drops of Ferri reagent, Weigert B. Gently stir and let it react.	3-5 minutes
6.	Rinse under tap water	1 min
7.	Add Phosphotungstic acid, 1% solution (≥5 drops)	3 min
8.	Rinse shortly in distilled water	
9.	Stain using Ladewig's reagent	4 min
10.	Rinse in distilled water	until the excessive dye is washed off of the section
11.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
12.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
13.	Dehydrate using 100% alcohol (Histanol 100)	2 min
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 VitroGnost cover glass.

b) 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 Add Phosphotungstic acid, 1% solution	3 min
9.	Rinse shortly in distilled water	
10.	Immerse in Ladewig's 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 - red 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 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 Ladewig's Trichrome kit in a tightly sealed original packaging at temperature of 15 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.

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Refer to the supplied documentation	Storage temperature range	Number of tests in package	REF	Product code	CE	European Conformity	BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com	CE
Refer to supplied instructions	Keep away from heat and sunlight	Valid until	LOT	Lot number		Manufacturer		
IVD For *in vitro* diagnostic use only	Keep in dry place	Caution - fragile						