

HEMATOXYLIN W KIT

IVD *In vitro* diagnostic medical device CE

Acid-resistant hematoxylin according to Weigert Two-reagent kit, stains nuclei blue-black

INSTRUCTIONS FOR USE

REF Product code: HEMW-100T (for 100 tests) HEMW-K-100 (2 x 100 mL) HEMW-K-500 (2 x 500 mL) HEMW-K-1L (2 x 1000 mL)

Introduction

Hematoxylin acc. to Weigert is used in combination with various special (trichrome) stains. Because of its resistance to acid solutions, it retains the dye and stains nuclear membranes. Unlike the standard hematoxylin used in histology, such as hematoxylin acc. to Harris, Mayer, Mayer-Lillie, and Gill, Hematoxylin acc. to Weigert contains ferric ions that create resistance to acid solutions and sudden pH value changes. Hematoxylin acc. to Weigert is most commonly used in the following trichrome methods: A.F.O.G., Gomori Trichrome, Masson-Goldner Trichrome, Masson Trichrome, Van Gieson Trichrome, Elastica-Van Gieson and Weigert-Van Gieson. Most of those methods are used for staining muscle and connective fibers.

Product description

- **HEMATOXYLIN W KIT** – Kit for staining nuclei

The kit contains:	for 100 tests (HEMW-100T)	2 x 100 mL (HEMW-K-100)	2 x 500 mL (HEMW-K-500)	2 x 1000 mL (HEMW-K-1L)
Hematoxylin, Weigert A	30 mL (HEMA-OT-30)	100 mL (HEMA-OT-100)	500 mL (HEMA-OT-500)	1000 mL (HEMA-OT-1L)
Ferri reagent, Weigert B	30 mL (FR-OT-30)	100 mL (FR-OT-100)	500 mL (FR-OT-500)	1000 mL (FR-OT-1L)

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 56/58, 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 C, or 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
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade
- Special stains for histology, such as A.F.O.G., Gomori Trichrome, Masson-Goldner Trichrome, Masson Trichrome, Movat, Mucicarmine, Van Gieson Trichrome, Elastica-Van Gieson and Weigert-Van Gieson kits

Preparation of working solution

Hematoxylin Weigert working solution

- Mix Hematoxylin, Weigert A and Ferri reagent, Weigert B in 1:1 ratio. Prepare a volume of working solution to be used with the staining procedure because the working solution is stable for about 2 weeks. Discard the solution when the nuclei turn brown after staining.

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 embed 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 (HEMW-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.	Stain with Hematoxylin Weigert working solution (add ≥5 drops)	5 – 10 minutes
	Note: for more strongly stained nuclei, incubate the sections for 10 minutes in Hematoxylin Weigert working solution	
6.	Rinse under tap water	3 min
7.	Stain with contrast reagent (depending on the type of special stains kit being used)	
8.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
9.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
10.	Dehydrate using 100% alcohol (Histanol 100)	2 min
11.	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 two-reagent 100 mL, 500 ml or 1000 ml kit (HEMW-K-100, HEMK-K-500, HEMW-K-1L)

Pour the reagents into staining jars (Coplín, Hellendahl, or Schifferdecker) and pour them back into the original bottles (discard the working solution or use it in 2 weeks' time). 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.	Immerse into Hematoxylin Weigert working solution	5-10 minutes
	Note: Note: for more strongly stained nuclei, incubate the sections for 10 minutes in Hematoxylin Weigert working solution	
6.	Rinse under tap water	3 min
7.	Stain with contrast reagent (depending on the type of special stains kit being used)	
8.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
9.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
10.	Dehydrate using 100% alcohol (Histanol 100)	2 min
11.	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

Nuclei - blue-purple (if the slide is stained only with Hematoxylin acc. to Weigert). Combined with special staining kits, nuclei turn blue-black

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 Hematoxylin W kit in a tightly sealed original packaging at temperature of +15 to +25°C Do not keep in cold 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. Bancroft, J.D. et Stevens, A. (1982): Theory and practice of histological techniques, 2nd ed., Churchill Livingstone, Edinburgh & London, UK.
2. Culling, C.F.A.(1974): Handbook of histopathological and histochemical techniques, 2nd ed., Butterworth, London, UK
3. Sheehan D.C. et Hrapchak, B.B.(1980): Theory and Practice Histotechnology, 2nd ed., CV Mosby, St. Louis, (MO), pp 52, p 14-167.

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	Refer to the supplied documentation		Storage temperature range		Number of tests in package		Product code		European Conformity	BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com	
	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						