BIOWAX 56/58

IVD *In vitro* diagnostic medical device CE

Blend of paraffin granules for optimal infiltration and routine work
INSTRUCTIONS FOR USE

REF Catalogue number: BW56/58-1 (1 kg) BW56/58-2 (2 kg) BW56/58-10 (10 kg)

Introduction:

Paraffin is the most commonly used medium for infiltration, embedding and creating tissue blocks. It enables processing of large amounts of tissue samples in a short period of time; serial samples can be easily obtained. Routine staining, but also the most complicated ones can be done simply. BioGnost's BioWax 56/58 paraffin is an optimal blend of paraffin wax and plastic polymers without added dimethyl sulfoxide (DMSO). Its main characteristics are low-level shrinking during cooling and ideal properties during cutting that provide optimal results during strips formation. Enables excellent infiltration into tissues and reduces the tendency of tissue to crack. Its granulated form allows easier handling and faster melting with melting point at 54°C/57°C. It is used for permeating tissue and forming paraffin blocks that enables creating paraffin sections. Sections can then be stained using standard histological techniques or use in different immunohistochemical and molecular-biological reactions.

Product description:

- **BIOWAX 56/58** – Blend of paraffins for use in histopathology.

Other preparations and reagents that may be used:

- Fixative such as BioGnost's neutral buffered formalin: 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
- 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, BioMount Aqua
- High-quality glass slides for use in histopathology, such as VitroGnost SUPER GRADE or one of more than 30 types of BioGnost's glass slides
- VitroGnost cover glass

Preparing the sample for embedding

- Fix the tissue sample well (Formaldehyde NB 4% or Formaldehyde NB 10%).
- Wash the sample thoroughly under a jet of water.
- Dehydrate the sample through series of ascending alcohol solutions (Histanol 70, Histanol 80, Histanol 95 and Histanol 100). Each exchange should last for 1 hour.
  Note: Dehydration of the sample in alcohol solution of the highest concentration (Histanol 100) is carried out through three exchanges, each lasting for 1 hour.
- Clear the tissue sample in xylene (BioClear) or in a xylene substitute (BioClear New) by two exchanges for 1 hour each.
  Note: Treating the sample with xylene enables paraffin to be completely infiltrated into the tissue and makes cutting paraffin blocks easier.
- Melt the paraffin (BioWax 56/58) in a thermostat at temperature of +59°C.
  Note: Histology cassettes (in which the tissue was dehydrated) are used for embedding.

Embedding the tissue sample into paraffin

- Fill the histology mold with melted paraffin, press the sample into the bottom of the mold and cover it with BioGnost's plastic histology cassette.
  Note: Liquid paraffin permeates the sample without causing thermal and chemical damage.
- Infiltration and embedding are being conducted in thermostat at temperature of +59°C in two exchanges (first exchange lasts for 2 hours, the second exchange lasts for 3 hours)
- Quickly cool the paraffin blocks in order to prevent building up of crystals in the sample.
  Note: Rapid cooling makes paraffin solidify on the inside and around the fixated sample. That enables cutting it into blocks.
- Fixate the blocks onto the pad (wooden plate or a metal pad).
- Cut the paraffin block on 4-6 μm thick slices.
  Note: If dehydration and clearing processes were incomplete, the paraffin blocks will be soft and unfit for cutting.
- By using a brush transfer the section onto the surface of distilled water heated to +45°C and mount onto VitroGnost glass slide.
  Note: Hot water will cause the section to be straightened and more easily mounted on a slide.
- Dry the section by air, put it into thermostat at temperature of +56°C and incubate for 12 hours.
- Apply the appropriate type of covering medium (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount C if BioClear xylene was used; use BioMount New or BioMount New Low in case BioClear New was used).
- Cover the section with a VitroGnost cover glass.

Result

After embedding the section into BioWax 56/58 paraffin, it is ready for further histochemical processing. Because of its exceptional characteristics, BioWax 56/58 enables optimal embedding of the sample without the block cracking or crumbling. It also enables excellent conservation of cellular and tissue structures, as well as long-term storage.

Note:

Duration of preparation and treatment of the sample is not standardized. Depending on personal requests and standard laboratory operating procedures, sample processing and embedding into paraffin can be carried out according to other protocols. Paraffin granules in time get glued together, but paraffin remains suitable for use. In that case, tap the closed bag of paraffin against hard surface before use.

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. In order to avoid an erroneous result, a positive and negative check is advised before application.

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 BioWax 56/58 in a tightly closed original package at temperature between +15°C and +25°C. Do not keep in cold places, do not freeze and avoid exposing to direct sunlight. Expiry date is stated on the product's label.

References

1. Carson, F. L., Hladik, C. (2009): *Histotechnology: A Self-Instructional Text*, 3rd ed., Chicago: ASCP Press
2. Kiernan, J. A. (2008): *Histological and Histochemical Methods*, 4th ed., Bloxham: Scion Publishing Ltd.

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	Refer to the supplied documentation	°C	Storage temperature range	Σ	Number of tests in package	REF	Product code	CE	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	Lot number		Manufacturer		
IVD	For *in vitro* diagnostic use only		Keep in dry place		Caution - fragile					CE	