

# **MUCICARMINE KIT**

IVD In vitro diagnostic medical device

## Four-reagent kit for determining acid mucopolysaccharides

## INSTRUCTIONS FOR USE

REF Catalogue number: MUC-100T (4x30 mL) MUC-K-100 (5x100 ml)

#### Introduction

Mucicarmine kit is used for visualizing acid mucopolysaccharides (mucins), which can be very useful when diagnosing the type of tumor (according to the tumor mucin secretion). It is also used in microbiology for identifying microorganisms based on cellular membrane coloration, but that is limited to the microorganisms that have complete cellular membrane (or partially) consisting of polysaccharides.

### **Product description**

MUCICARMINE KIT - Four-reagent kit for determining mucins

The kit contains:	100 tests (MUC-100)	5 x 100 ml (MUC-K-100)
Hematoxylin, Weigert A	30 ml (HEMA-OT-30)	100 ml (HEMA-OT-100)
Ferri reagent, Weigert B	30 ml (FR-0T-30)	100 ml (FR-0T-100)
Mayer's mucicarmine	30 mL (MMU-0T-30)	2x100 mL (MMU-0T-100)
Metanil yellow, solution	30 mL (MY-0T-30)	100 mL (MY-0T-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 52/54, 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, BioMount DPX, 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 tissue sample (4% NB Formaldehyde, 10% NB Formaldehyde), 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 52/54, BioWax Plus 56/58, BioWax 56/58, BioWax Blue, BioWax Micro).

Cut the paraffin block to 4-6  $\mu$ m 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 (MUC-100T)

Note: discard the working solution of mucicarmine after usage

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 using Hematoxylin, Weigert A (add 5 drops) and Ferri reagent, Weigert B (add 5 drops). Gently mix on the section and leave for incubation	2-5 minutes
6.	Rinse under tap water	10 min
7.	Prepare the working solution of mucicarmine: mix 5 drops of Mayer's mucicarmine with 0.5 mL of distilled (demi) water and add to the section. In order to avoid drying of the section, use incubation container/chamber	60 min
	Note: in case of staining bigger amounts of sections, prepare the working solution by mixing Mayer's mucicarmine with distilled (demi) water (1+2 dilution).  (example: 5 mL of Mayer's mucicarmine mix with 10 mL of distilled (demi) water)	
8.	Rinse in distilled (demi) water	
10.	Add Metanil yellow, solution (≥5 drops)	1 min (5 minutes when staining lungs)
11.	Rinse in distilled (demi) water	(skip this step when staining lungs)
12.	Dehydrate using 70% alcohol (Histanol 70)	5 dips (2 quick dips when staining lungs)
13.	Dehydrate using 95% alcohol (Histanol 95)	5 dips (2 quick dips when staining lungs)
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 a VitroGnost cover glass.

#### b) using four-reagent 100 mL kit (MUC-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. Discard the working solution of mucicarmine after usage

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Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
Rehydrate using 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
Rehydrate using 95% alcohol (Histanol 95)	2 min
Rehydrate in distilled (demi) water	2 min
Prepare 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	
Immerse into Weigert hematoxylin working solution	2-5 minutes
Rinse under tap water	10 min
Preparation of mucicarmine working solution: mix Mayer's mucicarmine with distilled (demi) water in 1+2 dilution, for instance 10 mL of Mayer's mucicarmine with 20 mL of distilled (demi) water. Dip and incubate the sections in the prepared working solution	60 min
Rinse in distilled (demi) water	
Immerse into Metanil yellow, solution.	1 min (5 minutes when staining lungs)
Rinse in distilled (demi) water	(skip this step when staining lungs)
Dehydrate using 70% alcohol (Histanol 70)	5 dips (2 quick dips when staining lungs)
Dehydrate using 95% alcohol (Histanol 95)	5 dips (2 quick dips when staining lungs)
Dehydrate using 100% alcohol (Histanol 100)	2 min
Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 2 min each
	Rehydrate using 100% alcohol (Histanol 100) Rehydrate using 95% alcohol (Histanol 95) Rehydrate in distilled (demi) water Prepare 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 Immerse into Weigert hematoxylin working solution Rinse under tap water Preparation of mucicarmine working solution: mix Mayer's mucicarmine with distilled (demi) water in 1+2 dilution, for instance 10 mL of Mayer's mucicarmine with 20 mL of distilled (demi) water. Dip and incubate the sections in the prepared working solution Rinse in distilled (demi) water Immerse into Metanil yellow, solution. Rinse in distilled (demi) water Dehydrate using 70% alcohol (Histanol 70) Dehydrate using 95% alcohol (Histanol 95) Dehydrate using 100% alcohol (Histanol 100)

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 a VitroGnost cover glass.

## Result

Nuclei - black

Acid mucopolysaccharides (mucins) - various hues of red

Other structures, neutral mucins - bright yellow

#### 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 Mucicarmine kit in a tightly closed original package at temperature between +15 °C and +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. Melis, M., Carpino, F., Di Tondo, U. (1989), Tecniche in anatomia patologica, Edi Ermes, Milano.
- 2. Prophet, E.B., Mills, B., Arrington, J., Sobin, L. (1968), Laboratory methods in histotechnology, McGraw Hill, Washington D.C.
- 3. Bancroft, J.D., Gamble, M. (2002), Theory and practice of Histological Techniques, Churchill Livingstone, New York.

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