

TOLUIDINE BLUE, SOLUTION

IVD *In vitro* diagnostic medical device

CE

Solution for nuclear staining and metachromatic staining mastocytes in connective tissues INSTRUCTIONS FOR USE

REF Catalogue number: TB-OT-100 (100 mL) TB-OT-250 (250 mL)

Introduction

Toluidine Blue, solution is often used for metachromatic staining (characteristic for cation or basic dyes) that partially depends on the pH level, dye concentration and temperature. During staining blue or purple dyes display a shift into red, while red dyes display shift into yellow on metachromatic tissue elements. Metachromatic staining is typical for mucopolysaccharides (especially for those with numerous sulfate groups), cartilage, cylindrical cells and mast cell granules.

Product description

- TOLUIDINE BLUE, SOLUTION** – 1% Toluidine blue solution for nuclear staining and metachromatic mast cell staining

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, 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 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 histological sections for staining

- Fix the tissue sample tightly (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 μ m slices and place them on a VitroGnost glass slide.

Sample staining procedure; nuclear staining / general sample review

Note: while staining cytology samples, staining procedure starts at rehydration with 95% alcohol (Step 3)

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Start rehydration by 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.	Rinse in distilled water	1 min
6.	Stain using Toluidine Blue, solution	10-20 minutes
7.	Rinse in distilled water	1 min
8.	*Dehydrate using 95% alcohol (Histanol 95)	10 dips
9.	**Dehydrate using 100% alcohol (Histanol 100)	2 exchanges, 10 dips each
10.	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.

*If permanent section is not necessary, add water-based covering medium (BioMount Aqua) on the section after rinsing in distilled water and cover with cover glass.

**Prolonged exposure to alcohol causes rinsing of Toluidine Blue off the section.

Result

Nuclei - dark blue

Tissue - blue

Sample staining procedure; metachromatic staining

Note: while staining cytology samples, staining procedure starts at rehydration with 95% alcohol (Step 3)

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.	Rinse in distilled water	1 min
6.	Stain using Toluidine Blue, solution or stain in diluted 0.01% Toluidine Blue, solution*	1-2 minutes 5-10 minutes
7.	Rinse in distilled water	1 min
8.	**Dehydrate using 95% alcohol (Histanol 95)	10 dips
9.	***Dehydrate using 100% alcohol (Histanol 100)	10 dips in 2 exchanges
10.	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.

*Mix 1 ml of Toluidine Blue, solution with 99 ml of distilled/demineralized water.

**If permanent section is not necessary, add water-based covering medium (BioMount Aqua) on the section after rinsing in distilled water and cover with cover glass.

***Prolonged exposure to alcohol causes rinsing of Toluidine Blue off the section.

Result

Nuclei and cytoplasm - (orthochromatic staining) blue

Various acid carbohydrates* - (metachromatic staining) pink to red or purple

* Connective tissues, mucins, basic substances of cartilage, mast cell granules and many epithelial mucins are stained metachromatically.

Note

Time periods of staining procedures are not standardized. Intensity of staining depends on the period of immersion in the dye. Real staining protocol depends on personal requests and standard laboratory operating procedures.

Preparing the sample and diagnostics

Use only appropriate instruments for collecting and preparing the samples. All the samples must be processed with the most modern technology and be visibly marked. Follow the manufacturer's instructions for handling. In order to avoid mistakes, staining must be conducted by a trained professional. Only trained medical personnel may make a diagnosis. 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 which is available on demand.














Storing, stability and expiry date

Keep Toluidine Blue, solution 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. Date of manufacture and expiry date are printed on the product's label.

References

1. Scott, S. G. (1912): On successive double staining for histological purposes Journal of Pathology and Bacteriology, v. 16, p. 390-398.
2. Sheehan, D.C. et Hrapchak, B.B. (1980): Theory and Practice of Histotechnology, 2nd ed St. Louise: CV Mosby Co.
3. Kiernan J. A. (2008) Histological and histochemical methods, 4th ed. Bloxham: Scion Publishing Ltd.

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 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 <i>in vitro</i> diagnostic use only	 Keep in dry place	 Caution - fragile		

 BIOGNOST Ltd.
Medjugorska 59
10040 Zagreb
CROATIA
www.biognost.com

