

# TOLUIDINE BLUE, SOLUTION



IVD In vitro diagnostic medical device

Classified acc. to Regulation (EU) 2017/746 - Class A device

## Solution for nucleus staining and metachromatic staining of mastocytes in connective tissue

### INSTRUCTIONS FOR USE

Basic UDI number	385889212HPC30708STARVF		
EMDN code	W01030708		
REF	Catalog number	Volume	UDI-DI number
TB-OT-100		100 mL	03858888824754
TB-OT-250		250 mL	03858888824761



#### Intended use and test principle

Toluidine Blue solution is often used for metachromatic staining, characteristic for cationic or alkaline dyes, which partially depends on pH, dye concentration and temperature. pH. Using this method, blue or purple dyes change into red and red dyes change into yellow on metachromatic tissue elements. Metachromatic staining is typical for mucopolysaccharides (especially those with numerous sulfate groups), cartilage, cylindric cells and mastocyte granules.

#### Product description

- **TOLUIDINE BLUE, SOLUTION** – 1% Toluidine blue solution for staining nuclei and metachromatic mastocyte staining

#### Additional reagents and materials that can be used in staining:

- Fixative agents such as BioGnost's neutral buffered formaldehyde solutions: Formaldehyde NB 4%, Formaldehyde NB 10%
- Dehydration/rehydration agents such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95, and Histanol 100
- Clearing agents, such as BioClear xylene or BioClear New, an aliphatic hydrocarbon-based xylene substitute
- Infiltration and embedding agents such as BioGnost's granulated paraffins BioWax 52/54, BioWax 56/58, BioWax Plus 56/58, BioWax Blue
- Microscopic slide covering agents and cover glass mountants such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount DPX New, BioMount C, BioMount Aqua
- VitroGnost slides and coverslips for use in histopathology and cytology
- BioGnost's immersion oils, types A, C, FF, 37 or Tropical Grade

#### Preparation of histological sections for staining

- Fix (Formaldehyde NB 4%, Formaldehyde NB 10%) and process the tissue sample
- Embed the tissue in a paraffin block (BioWax 52/54, BioWax 56/58, BioWax Plus 56/58, BioWax Blue)
- Cut the paraffin block into 4-6  $\mu\text{m}$  thin slices and mount on a VitroGnost microscope slide

#### NOTE

Apply the reagent so it completely covers the section.

When staining cytological samples, start the procedure from rehydration in 95% alcohol (step 3).

If a permanent slide is not required, after step 7 apply a water-based mounting medium (BioMount Aqua) and apply the coverslip.

Prolonged exposure to alcohol causes the washing out of Toluidine Blue stain from the preparation

#### Sample staining procedure – nucleus staining

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate in 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled/demineralized water	2 min
5.	Rinse in distilled/demineralized water	1 min
6.	Stain with Toluidine Blue, solution	10-20 min
7.	Rinse in distilled/demineralized water	1 min
8.	Dehydrate in 95% alcohol (Histanol 95)	10 dips
9.	Dehydrate in 100% alcohol (Histanol 100)	2 exchanges with 10 dips
10.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium. If BioClear xylene was used, use one of BioGnost's xylene-based mountants (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate mountant is BioMount New. Cover the section with a VitroGnost cover glass.

#### Sample staining procedure – metachromatic staining

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 exchanges, 2 min each
2.	Rehydrate in 100% alcohol (Histanol 100)	2 exchanges, 5 and 3 min
3.	Rehydrate in 95% alcohol (Histanol 95)	2 min
4.	Rehydrate in distilled/demineralized water	2 min
5.	Ispiranje u destiliranoj/demineraliziranoj vodi	1 min
6.	Stain with Toluidine Blue, solution	1-2min
	Or stain with diluted 0.01% Toluidine Blue solution*	5-10 min
7.	Rinse in distilled/demineralized water	1 min
8.	Dehydrate in 95% alcohol (Histanol 95)	10 dips
9.	Dehydrate in 100% alcohol (Histanol 100)	10 dips in 2 exchanges
10.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges, 2 min each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium. If BioClear xylene was used, use one of BioGnost's xylene-based mountants (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate mountant is BioMount New. Cover the section with a VitroGnost cover glass.

\* Mix 1 mL of Toluidine Blue, solution and 99 mL of distilled/demineralized water

### Result

Nuclei and cytoplasm – blue

Connective tissue, mucins, base substance of cartilage, mastocyte granules, numerous epithelial mucins – pink to red or purple

### Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations in the preparation of the staining solution and from the staining procedure described in BioGnost's instructions for use may cause variations in the results. Prolonged exposure of the stained slide to alcohol during the dehydration process causes the washing out of the Toluidine Blue stain from the slide.

### Sample preparation and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples using modern technology and mark them clearly. Be sure to follow the manufacturer's handling instructions. To avoid errors, staining and diagnosis can only be carried out by qualified personnel. Use a microscope equipped according to medical diagnostic laboratory standards.

### Safety at work and environmental protection

Handle the product in accordance with occupational health and environmental protection guidelines. Used and expired solutions must be disposed of as special waste following national guidelines. Reagents used in this procedure can pose a danger to human health. The examined tissue samples are potentially infectious, and it is necessary to take the measures needed to protect human health in accordance with the guidelines of good laboratory practice. It is mandatory to read and act according to the information and warning signs printed on the product label and in the Safety Data Sheet, which is available on request.

If a serious incident occurs during use of this product or as a result of its use, please report it to the manufacturer or authorized representative and competent authority.

### Storage, stability, and shelf life

Upon receipt, store the product in a dry place and well-closed original packaging at a temperature of +15 °C to +25 °C. Do not freeze or expose to direct sunlight. After first opening, the product can be used until the specified expiry date, if stored properly. The expiration date is printed on the product 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. i 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.

### Warnings and precautions regarding the materials contained in the product:

Not a hazardous substance or mixture acc. to Regulation (EZ) no. 1272/2008.

TB-IFU\_ENV5, 13.01.2026., IŠP

 Manufacturer	 Batch code	 Consult instructions for use	 European conformity
 Date of manufacture	 Catalogue number	 Caution	 Unique device identifier
 Use-by date	 Temperature limit	 <i>In vitro</i> diagnostic medical device	

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Version	Description / reason for change	Date
5	Revised in acc. to Regulation (EU) 2017/746 - IVDR	13.01.2026.