BIOGNOST®

ANILINE BLUE, C.I. 42780

CE IVD *In vitro* diagnostic medical device Classification according to Regulation (EU) 2017/746 - **Class A** device

For the selective staining of collagen and connective tissue in trichrome methods

China Blue, Acid Blue 93, C.I. 42780

INSTRUCTIONS FOR USE

Basic UDI-DI	385889212HPC30707PDYETD	
EMDN code	W01030707	
REF **Catalog number**	**Mass**	**UDI-DI**
CAB-P-25	25 g	03858888820329

Intended use and test principle

Histology, cytology, and other related scientific disciplines study the microscopic anatomy of tissues and cells. Proper staining is required to achieve good visualization of tissue and cellular structures. Aniline Blue dye, also known as China Blue, is most commonly used in trichrome staining methods (such as Masson's Trichrome) and is primarily used for staining collagen fibers blue. Aniline Blue dye is an anionic (negatively charged) dye that binds to positively charged groups in the tissue through electrostatic interactions. Trichrome staining methods use dyes of different molecular sizes. Larger Aniline Blue molecules penetrate densely packed structures, such as the cytoplasm, with difficulty, but are well retained in collagen fibers and connective tissue. After staining the sample, acids (most commonly phosphomolybdic or phosphotungstic acid) are used to remove previously bound dyes from collagen and create conditions for Aniline Blue to bind selectively to collagen fibers only. Collagen contains a large number of basic groups that allow binding of the acidic dye. As a result, collagen fibers stain intensely blue, whereas muscles, cytoplasm, and erythrocytes are stained by other dyes used in the trichrome method. Thus, the selectivity of Aniline Blue is not due to a specific chemical reaction with collagen, but rather to a combination of electrostatic binding, dye molecule size, and acid differentiation, resulting in preferential staining of collagen fibers. Numerous staining methods incorporate Aniline Blue dye as part of the staining procedure. Well-known examples include the Gomori method, in which Aniline Blue is used in combination with Chromotrope 2R dye; Heidenhain's AZAN stain and Mallory's polychrome stain (both of which contain an Aniline Blue-Orange G mixture as part of the staining method); Mann's stain (containing an Aniline Blue-Eosin mixture); Masson's stain and Lendrum's MSB technique, which use Aniline Blue in the final staining step; and Mallory's connective tissue stain, in which Aniline Blue is used in combination with Fuchsin Acid dye.

Product description

- **ANILINE BLUE, C.I. 42780** - powder dye for the preparation of a staining solution for the microscopic identification of connective tissue and collagen

Example of the use of Aniline Blue powder dye, C.I. 42780, in the Gomori trichrome staining method

Additional reagents and materials that can be used in the method

- Fixatives, 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
- Immersion oils such as BioGnost's Immersion Oil, Immersion Oils types A, C, FF, 37, or Immersion Oil Tropical Grade
- Chemicals such as: glacial acetic acid (CH_3COOH) and phosphotungstic acid ($H_3PW_{12}O_{40}{\cdot}xH_2O$)
- BioGnostova's powder dye for microscopy Chromotrope 2R
- Remaining components of the Gomori trichrome kit: Bouin's solution, Hematoxylin, Weigert A, Ferri reagent, Weigert B, and Differentiation reagent in the Gomori trichrome kit

Preparation of staining solution Aniline Blue/Chromotrope 2R (100 mL)

- Dissolve 0.8 g of phosphotungstic acid in 100 mL of distilled/demineralized water. Add 0.6 g of Chromotrope 2R powder dye and 0.3 g of Aniline Blue powder dye, and stir until completely dissolved. Add 1 mL of glacial acetic acid to the dye solution. Mix thoroughly. Store in a tightly closed bottle at room temperature until use.

Preparation of histological sections for staining

- Fix the tissue sample (Formaldehyde NB 4%, Formaldehyde NB 10%) and process
- 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 micron thin sections and mount on a VitroGnost microscope slide

Suggested staining procedure according to the Gomori trichrome method

Pour the reagents into glass staining jars (type Coplin, Hellendahl or Schifferdecker) and return them to the original bottles after staining. Close well. If necessary, filter the reagents.

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.	Stain using Bouin's solution	60 minutes at 56°C or overnight at room temperature
6.	Cool the section down to room temperature	10 min
7.	Rinse under running tap water	10 sec
8.	Rinse in distilled/demineralized water	10 sec
	Prepare the Weigert hematoxylin working solution: mix an equal volume of Hematoxylin, Weigert A and Feri reagent, Weigert B. Note: The working solution is stable for approximately two weeks. Prepare a volume of the working solution sufficient for staining the test preparations	

9.	Immerse into Weigert hematoxylin working solution and let it react	5 - 10 min
10.	Rinse under running tap water	5 min
11.	Immerse in Chromotrope 2R/Aniline Blue staining solution	3-5 min
12.	Rinse in distilled/demineralized water	5 dips
13.	Immerse in Differentiation reagent of the Gomori trichrome kit.	2 min
14.	Rinse in distilled/demineralized water	5 dips
15.	Dehydrate in 70% alcohol (Histanol 70)	5 dips
16.	Dehydrate in 95% alcohol (Histanol 95)	5 dips
17.	Dehidracija u 100%-tnom alkoholu (Histanol 100)	2 min
18.	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.

Result

Collagen - blue
Muscle fiber - pink
Nuclei - red to purple
Erythrocytes - yellow to orange

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the method of preparing the dye solution or from the staining procedure may cause variations in the staining results shown in this Instructions for Use.

Sample preparation and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples using modern technology and mark them clearly. It is necessary to follow the manufacturer's instructions for use. To avoid errors, sample preparation, staining, and diagnosis may only be performed by qualified personnel. Use a microscope that complies with medical diagnostic laboratory standards.
If a serious incident occurs during use or as a result of its use, please report it to the manufacturer and/or authorized representative and the competent authority.

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, therefore it is necessary to implement human health protection measures in accordance with good laboratory practice guidelines. 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.

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 production date and expiration date are printed on the product label.

References

1. Conn, J. (1977): *Biological Stains*, 9th ed., Baltimore: Williams and Wilkins Co.
2. Mallory, F. B. (1938): *Pathological techniques*, Philadelphia, W.B. Saunders Company
3. Gomori, G. (1950): *Aldehyde fuchsin: A new stain for elastic tissues*, American Journal of Clinical Pathology 20, 665.

Warnings and precautions regarding the materials contained in the product:

H302	Harmful if swallowed.
H315	Causes skin irritation.
H319	Causes serious eye irritation.
H335	May cause respiratory irritation.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302+P352	IF ON SKIN: wash with plenty of water.
P305+P351+P338	IF IN EYES: rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing.
P308+P313	IF exposed or concerned: Get medical advice/attention.

CAB-IFU_ENV4, 21.05.2026., IŠP

	Manufacturer	LOT	Batch code		Consult Instructions for use	CE	European conformity
	Date of manufacture	REF	Catalogue number		Caution	UDI	Unique device identifier
	Use-by date	°C	Temperature limit	IVD	*In vitro* diagnostic medical device		

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Version	Description / reason for change	Date
4	Revised acc. to Regulation (EU) 2017/746 - IVDR	21.05.2026.