ANILINE BLUE REAGENT

IVD In vitro diagnostic medical device CE

For use in Masson Trichrome kit

INSTRUCTIONS FOR USE

REF Product code: ABR-OT-100 (100 mL)

Introduction

Aniline Blue reagent is a component of Masson Trichrome kit used for visualization of muscles, collagen fibers and connective tissues, gametes, nuclei, neurofibrils, neuroglia, collagen, keratin intracellular fibrils and negative visualization of the Golgi apparatus. It is a method of staining muscle and collagen fibers in tissues during which Aniline Blue dye binds with collagen making it turn distinct blue. It is also used for visualization of increased collagen build up associated with functioning tissue being mistaken for scar tissue (liver sclerosis diagnosis), but also for differentiating smooth muscle fibers and collagens.

Product description

- **ANILINE BLUE REAGENT** - Reagent for staining collagen fibers.

Example of use of Aniline Blue reagent as a component of Masson Trichrome kit

Other slides 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 56/58, BioWax 56/68, BioWax Blue, BioWax Micro
- Glass slides used in histology, pathology and cytology, such as VitroGnost SUPER GRADE or VitroGnost COLOR , or one of 30 (and more) BioGnost's glass slides
- 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 New, BioMount C, and Canada Balsam
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types 37, A, C, FF, Tropical Grade, Cedarwood oil
- Other reagents of Masson Trichrome kit: Bouin's solution, Hematoxylin, Weigert A, Ferri reagent, Weigert B, Biebrich Scarlet-Acid Fuchsin reagent, P.T.A.-P.M.A. reagent, Acetic acid, 1% solution

Preparing the 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 Plus 56/58, BioWax 56/58, BioWax Blue, BioWax Micro)
  Cut the paraffin block to 4-6 μm slices and place them on a VitroGnost glass slide

Section staining procedure using 100 ml seven-reagent kit

Pour the reagents into glass staining jars (Coplin, Hellendahl or Schifferdecker), return to original bottles after staining. Close tightly. Filter the reagents if necessary.

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.	Immerse into Bouin's solution:	60 min at 56˚C or over night at room temperature
6.	Cool the sections down to room temperature	10 min
7.	Rinse under tap water	10 seconds
8.	Rinse in distilled water	10 seconds
9.	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	
10.	Immerse into Weigert hematoxylin working solution and let it react	5 min
11.	Rinse under tap water	3 min
12.	Immerse in Biebrich Scarlet-Acid Fuchsin reagent	2 min
13.	Rinse in distilled water	until the excessive dye is washed off of the section
14.	Immerse in PTA-PMA reagent	10 min
15.	Pour the reagent off the section without rinsing	
16.	Immerse in Aniline Blue reagent	5 min
17.	Rinse in distilled water	until the excessive dye is washed off of the section
18.	Immerse into Acetic acid, 1% solution	3 min
19.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
20.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
21.	Dehydrate using 100% alcohol (Histanol 100)	2 min
22.	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.

Result

Nuclei - black
Muscle fibers, keratin, cytoplasm - bright red
Collagen, mucus - blue
Erythrocytes - red-orange

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 Aniline Blue reagent in a tightly sealed original packaging at temperature of 15 to 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. Culling, C.F.A. (1974): Handbook of histopathological and histochemical techniques, 2nd ed., Butterworth, London, UK.
2. Lillie, R.D. (1945): Studies on selective staining of collagen with acid aniline dyes, J. Technical Methods, 25:1
3. Melis, M., Carpino, F., Di Tondo, U. (1989), Tecniche in anatomia patologica, Edi Ermes, Milano.
4. Sheehan D.C. et Hrapchak, B.B. (1980): Theory and Practice Histotechnology, 2nd ed., CV Mosby, St. Louis, (MO), pp 52, p 14-167.

ABR-OT-X, V3-EN2, 13 January 2017, AK/VR

Refer to the supplied documentation	Storage temperature range	Number of tests in package	REF	Product code	CE	European Conformity	BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com	CE
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						