BIOGNOST®

AZAN TRICHROME KIT

IVD *In vitro* diagnostic medical device CE

Five-reagent kit for staining connective tissue

INSTRUCTIONS FOR USE

REF Product code: AZT-100T (for 100 tests) AZT-K-250 (5x250 mL)

Introduction

Azan Trichrome kit is a modification of Mallory Trichrome kit for staining connective tissue. It is used for visualizing musles, collagen fibers, glial cells, glomerular cells, chromatins and erythrocytes of the same section. The kit contains two acid dyes: Azocarmine G and Aniline Blue counterstain. Azocarmine G is used at the beginning stage of the staining procedure, and Aniline Blue is used at the final stage, after the section is treated with phosphomolybdic acid. In order to achieve high quality staining results, it is necessary to stain the section using Azocarmine G, and then differentiate it progressively using aniline alcoholic solution in order to enable counterstaining of certain structures (such as collagen) of the section.

Product description

- **AZAN TRICRHOME KIT** - Five-reagent kit for staining connective tissue

The kit contains:	100 tests (AZT-100T)	5 x 250 mL (AZT-K-250)
Azocarmine, solution	100 mL (AZ-OT-100)	250 mL (AZ-OT-250)
Aniline, alcoholic solution	30 mL (ANA-OT-30)	250 mL (ANA-OT-250)
Acid alcohol, Azan	30 mL (KAA-OT-30)	250 mL (KAA-OT-250)
Phosphomolybdic acid, 5% solution	100 mL (FMK5-OT-100)	250 mL (FMK5-OT-250)
Azan reagent	100 mL (AZR-OT-100)	250 mL (AZR-OT-250)

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 agent, such as BioClear xylene or its aliphatic hydrocarbon substitutes, such as BioClear New
- 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 DPX, BioMount DPX High, BioMount DPX Low, BioMount C, BioMount Aqua, Canada Balsam or MountQuick Tube medium
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- BioGnost's immersion oils, such as Immersion oil, Cedarwood oil, Immersion oils types A and C

NOTE

- Apply the reagent so it completely covers the section.

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

Histological sections staining procedure

a) using kit for 100 tests (AZT-100T)

Pour the reagents (100 ml volumes) into glass staining jars (Coplin, Hellendahl or Schifferdecker), return to original bottles after staining. Heated Azocarmine solution cool down to room temperature and return to the original packaging. Close tightly.

1.	Deparaffinize the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3 exchanges, 10 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.	Heat Azocarmine solution to +56°C and immerse the sections. Cover the staining jar to avoid evaporation	30 min at +56 °C
6.	Cool the sections down at room temperature	5 min
7.	Rinse the section with tap water	until the excessive dye is washed off of the section (a few seconds)
8.	Add Aniline, alcoholic solution (≥ 5 drops) and differentiate	1 min
	Note: it is recommended to differentiate using microscopic examination of the section. Rinse the section with distilled (demi) water and examine the section microscopically. Repeat the step if necessary.	
9.	Add Acid alcohol, Azan (≥5 drops)	1 min
10.	Remove the reagent off the section without rinsing	
11.	Treat using Phosphotungstic acid, 5% solution: pour the solution into the staining jar and immerse the sections. Cover the staining jar to avoid evaporation	Treat intestinal sections and sections of small intestine (and similar tissues) for 30 minutes; treat the kidney sections for 60 minutes
12.	Remove the solution off the section without rinsing	
13.	Stain using Azan reagent: pour the reagent in the staining jar and immerse the sections Cover the staining jar to avoid evaporation	Stain intestinal sections and sections of small intestine (and similar tissues) for 30 minutes; treat the kidney sections for 60 minutes
14.	Rinse in distilled (demi) water	2 exchanges, 5 dips each
15.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
16.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
17.	Dehydrate using 100% alcohol (Histanol 100)	2 min
18.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 5 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 five-reagent 250 mL kit (AZT-K-250)

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, 10 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.	Heat Azocarmine solution to +56°C and immerse the sections. Cover the staining jar to avoid evaporation	30 min at +56 °C
6.	Cool the sections down at room temperature	5 min
7.	Rinse the section with tap water	until the excessive dye is washed off of the section (a few seconds)
8.	Immerse into Aniline, alcoholic solution and differentiate	1 min
	Note: it is recommended to differentiate using microscopic examination of the section. Rinse the section with distilled (demi) water and examine the section microscopically. Repeat the step if necessary.	
9.	Immerse into Acid alcohol, Azan	1 min
10.	Move to the next step without rinsing the section	
11.	Immerse into Phosphomolybdic acid, 5% solution Cover the staining jar to avoid evaporation	Treat intestinal sections and sections of small intestine (and similar tissues) for 30 minutes; treat the kidney sections for 60 minutes
12.	Move to the next step without rinsing the section	
13.	Immerse in Azan reagent. Cover the staining jar to avoid evaporation	Stain intestinal sections and sections of small intestine (and similar tissues) for 30 minutes; treat the kidney sections for 60 minutes
14.	Rinse in distilled (demi) water	2 exchanges, 5 dips each
15.	Dehydrate using 70% alcohol (Histanol 70)	5 dips
16.	Dehydrate using 95% alcohol (Histanol 95)	5 dips
17.	Dehydrate using 100% alcohol (Histanol 100)	2 min
18.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	2 exchanges, 5 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, erythrocytes, acidophilic granules of the pituitary gland - red
Neurofibrils (neuroglia) - hues of red
Muscle fibers - pink to red-pink
Collagen, reticulin, basophilic cell membranes, renal glomerular stroma, basement membranes - blue to dark blue
Elastic fibers - no color

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 Azan Trichrome kit 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. 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.

AZT-X, V3-EN3, 19 April 2016, IŠP/VR

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