

AZAN TRICHROME KIT



IVD In vitro diagnostic medical device

Classification according to Regulation (EU) 2017/746 – Class A device

Five-reagent kit for connective tissue staining acc. to Mallory

INSTRUCTIONS FOR USE

BASIC UDI-DI	385889212HPC30708STARVF		
EMDN code	W01030708		
REF	Catalog number	Volume	UDI-DI
AZT-100T		For 100 tests	03858890002942
AZT-K-250		5x250 mL	03858890005202



Intended use and test principle

The Azan Trichrome Kit is Heidenhain's modification of the Mallory trichrome kit for connective tissue staining. It is used for the visualization of muscle, collagen fibers, glial cells, glomerular stroma, chromatin, and erythrocytes on the same slide. The kit contains two acid dyes: Azocarmine and the counterstain Aniline Blue. In the first step, Azocarmine, as an anionic dye, selectively stains nuclei and cytoplasm depending on their affinity for acid dyes. After differentiation with Acid Alcohol and an alcoholic aniline solution, the next step involves the application of a phosphomolybdic acid solution, which removes the bound basic dye (Azocarmine) from the cytoplasm and collagen while leaving it firmly bound in the nuclei, thereby achieving clear nuclear contrast. In the final step, sections are stained with Azan reagent, which stains collagen and other connective tissue components blue, with nuclei stained red and cytoplasm and muscle stained red-pink.

Product description

- AZAN TRICHROME KIT** – Five-reagent kit for connective tissue staining

The kit contains:	100 tests (AZT-100T)	5 x 250 mL (AZT-K-250)	Storage temperature
Azocarmine, solution	100 mL (AZ-OT-100)	250 mL (AZ-OT-250)	15-25°C
Aniline, alcoholic solution	30 mL (ANA-OT-30)	250 mL (ANA-OT-250)	15-25°C
Acid Alcohol, Azan	30 mL (KAA-OT-30)	250 mL (KAA-OT-250)	15-25°C
Phosphomolybdic acid, 5% solution	100 mL (FMK5-OT-100)	250 mL (FMK5-OT-250)	15-25°C
Azan reagent	100 mL (AZR-OT-100)	250 mL (AZR-OT-250)	15-25°C

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
- A clearing agent such as BioClear xylene or a substitute such as BioClear New, an aliphatic hydrocarbon-based agent
- Infiltration and embedding agents such as BioGnost's granulated paraffins BioWax 52/54, 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 C, BioMount Aqua
- VitroGnost slides and coverslips for use in histopathology and cytology
- BioGnost's immersion media such as Immersion Oil, Immersion Oils types A, C, FF, 37, or Immersion Oil 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 micron thin sections and mount on a VitroGnost microscope slide

NOTE

Apply the reagent so that it completely covers the section.

Staining procedure for histological sections

a) using the 100-test kit (AZT-100T)

Pour the 100 mL reagents into staining jars (type Coplin, Hellendahl, or Schifferdecker) and return them to the original packaging after staining. Allow the heated Azocarmine solution to cool to room temperature before returning it to the original packaging. Close well.

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 exchanges, 10 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.	Heat the Azocarmine solution to +56°C and immerse the slides. Cover to prevent evaporation	30 min at +56 °C
6.	Allow slides to cool to room temperature	5 min
7.	Rinse in running tap water	until excess dye is rinsed from the section (a few seconds)
8.	Apply Aniline, alcoholic solution (≥ 5 drops) and differentiate	1 min
	Note: it is recommended to perform differentiation with microscopic control of the slide. After one minute, rinse the section in distilled/demineralized water and examine microscopically. If necessary, repeat the step	
9.	Apply Acid Alcohol, Azan (≥ 5 drops)	2-5 s
10.	Remove the reagent from the section without rinsing	
11.	Treatment with Phosphomolybdic acid, 5% solution: pour the solution into a staining jar and immerse the sections. Cover to prevent evaporation	Treat stomach and small intestine sections (gastrointestinal system sections) for 30 minutes, and kidney sections for 60 minutes
12.	Remove the solution from the slide without rinsing	
13.	Staining with Azan reagent: pour the reagent into a staining jar and immerse the sections. Cover to prevent evaporation	Stain stomach and small intestine sections (gastrointestinal system sections) for 5-10 minutes, and kidney sections for 20-25 minutes
14.	Rinse in distilled/demineralized water	2 exchanges of 5 dips
15.	Dehydrate in 70% alcohol (Histanol 70)	5 dips
16.	Dehydrate in 95% alcohol (Histanol 95)	5 dips
17.	Dehydrate in 100% alcohol (Histanol 100)	2 min
18.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges of 5 min each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium to the section. 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.

b) using the five-reagent 250 mL kit (AZT-K-250)

Pour the reagents into staining jars (type Coplin, Hellendahl, or Schifferdecker) and return them to the original bottles after staining. Allow the heated Azocarmine solution to cool to room temperature before returning it to the original packaging. Close well. Filter the reagents as needed.

1.	Deparaffinize in xylene (BioClear) or xylene substitute (BioClear New)	3 exchanges, 10 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.	Heat the Azocarmine solution to +56°C and immerse the sections. Cover to prevent evaporation	30 min at +56 °C
6.	Allow sections to cool to room temperature	5 min
7.	Rinse in running tap water	until excess dye is rinsed from the section (a few seconds)
8.	Immerse in Aniline, alcoholic solution and differentiate	1 min
	Note: it is recommended to perform differentiation with microscopic control of the section. After one minute, rinse the section in distilled/demineralized water and examine microscopically. If necessary, repeat the step	
9.	Immerse in Acid Alcohol, Azan	2 to 5 dips
10.	Without rinsing the sections, proceed to the next step	
11.	Immerse in Phosphomolybdic acid, 5% solution. Cover to prevent evaporation	Treat stomach and small intestine sections (gastrointestinal system sections) for 30 minutes, and kidney sections for 60 minutes
12.	Without rinsing the sections, proceed to the next step	
13.	Immerse in Azan reagent. Cover to prevent evaporation	Stain stomach and small intestine sections (gastrointestinal system sections) for 5-10 minutes, and kidney sections for 20-25 minutes
14.	Rinse in distilled/demineralized water	2 exchanges of 5 dips
15.	Dehydrate in 70% alcohol (Histanol 70)	5 dips
16.	Dehydrate in 95% alcohol (Histanol 95)	5 dips
17.	Dehydrate in 100% alcohol (Histanol 100)	2 min
18.	Clear in xylene (BioClear) or xylene substitute (BioClear New)	2 exchanges of 5 min each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium to the section. 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

Nuclei, erythrocytes, acidophilic pituitary granules: red

Neurofibrils (neuroglia): shades of red

Muscle fibers: pink to red-pink color

Collagen, reticulin, basophilic cell membranes, renal glomerular stroma, basement membranes: blue to dark blue

Elastic fibers: unstained

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in this Instructions for Use may cause differences in staining results.

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, the staining process and diagnosis can only be carried out by authorized and professionally 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 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, instructions for use 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

- Melis, M., Carpino, F., Di Tondo, U. (1989), Tecniche in anatomia patologica, Edi Ermes, Milan.
- Prophet, E.B., Mills, B., Arrington, J., Sobin, L. (1968), Laboratory methods in histotechnology, McGraw Hill, Washington D.C.
- Bancroft, J.D., Gamble, M. (2002), Theory and practice of Histological Techniques, Churchill Livingstone, New York.

Warnings and precautions regarding the materials contained in the product:

H225	Highly flammable liquid and vapour.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P233	Keep container tightly closed.
P280	Wear protective gloves/protective clothing/eye protection/face protection.

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



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Version	Description / reason for change	Date
5	Revised acc. to Regulation (EU) 2017/746 - IVDR	02.02.2026.