619-71

Microscopy

Harleco® Giemsa Stain Solution Azure B

IVD

In Vitro Diagnostic Medical Device

Intended Use

Harleco® Giemsa Stain Solution Azure B is a polychromatic stain used alone or in combination with Wright Stain for staining of blood smears. It is also used for clinical cell diagnosis and serves the purpose of investigating sample material of human origin.

Principle

Giemsa stain is a Romanowsky neutral dye stain based on the combination of methylene blue azure as the basic dye component and eosin as the acid dye component [1,2,3]. Romanowsky modified Ehrlich's earlier finding of a neutral dye which offered the ability to identify acidophilic, basophilic, and neutrophilic granules of leukocytes. The Harleco® Giemsa Azure B solution is formulated to provide optimal differential staining.

Sample material

Peripheral blood smears should be prepared from a freshly drawn blood specimen (EDTA purple top tubes). Allow smears to air dry completely before staining.

Reagent

Cat. No. 619 500 mL
Harleco® Giemsa Stain Solution Azure B

Also required:

Cat. No. 1217	Buffer, Phosphate pH 6.4	1 L, 4 L
or		
Cat. No. 1218	Buffer, Phosphate pH 6.8	1 L, 4 L
or		
Cat. No.1219	Buffer, Phosphate pH 7.0	1 L, 4 L
Cat. No. 6442	Water, Deionized Distilled, ASTM Type II	4 L, 20 L
Cat. No. 65044A	HemaColor® Stain Solution (fixative)	4 L
Cat. No. 740	Harleco® Wright Stain	1 L, 4 L, 10 L

Sample preparation

The sampling must be performed by qualified personnel. All samples must be clearly labeled. Suitable instruments must be used for collecting and preparing samples. Follow the manufacturer's instructions for application/use.

Reagent preparation (Stain-Buffer Mixture)

- The solution is supplied as a concentrated staining solution and must be diluted.
- Add 5 mL of Giemsa Stain to 45 mL of Phosphate Buffer of appropriate pH.
- Mix and allow to stand 10-15 minutes before use.
- The resulting Stain-Buffer Mixture is stable for 2 hours.

Manual Staining Procedure

1. Prepare fresh peripheral blood smears.
2. Allow to air dry completely before staining.
3. Place slide in methanol (fixative) (Cat. No. 65044A) for 1-2 minutes.
4. Place slide in Wright Stain (Cat. No. 740) for 4 minutes.
5. Place slide in Stain-Buffer Mixture (prepared as described above) for 8 minutes.
6. Drain Stain-Buffer Mixture and rinse with 10-15 mL of deionized water.
7. Air dry and examine microscopically under oil immersion.

Results

Cell Type	Nuclei	Granules	Cytoplasm
Erythrocytes		Yellowish Red	Yellowish Red
Polymorpho-nuclear Neutrophilic Leucocytes	Dark Blue to Purple	Reddish Lilac	Pale Pink
Basophilic Leucocytes	Purple or Dark Blue	Dark Purple to Black	
Eosinophilic Leucocytes	Blue to Purple	Red to Orange Red	Blue
Lymphocytes	Dark Purple		Sky Blue
Platelets		Violet to Purple	

Technical notes for Manual Staining Procedures

1. Experimentation and adjustment to staining times may be required to obtain optimal results and cell differentiation.
2. Best results are obtained when the following are observed:
   a. Slides are clean and free of grease and debris.
   b. Methanol fixative is acetone free.
   c. Blood smears are freshly prepared.
   d. Blood smears are prepared as a very thin layer on slide.
3. Staining intensity can be increased by extending the timing in steps 4 and 5. However this will only have moderate effects on the intensity.
4. pH 6.4 buffer will produce acidophilic results. RBCs will be pink in color. (step 5)
5. pH 6.8 buffer will produce neutrophilic results. RBCs will appear yellow-pink to tan. (step 5)
6. Distilled water will produce basophilic results. RBCs will appear gray to blue-gray. (step 5)
7. Best results for reading slide are at the feathered end of smear.

Diagnostics

Diagnoses are to be made only by authorized and trained personnel. Valid nomenclature must be used. Further tests must be selected and implemented according to recognized methods. Suitable controls should be conducted with each application.

Storage

15-25 °C

Shelf-life

The Giemsa Stain Solution Azure B for microscopy can be used until the stated expiry on the packaging.

After first opening of the bottle, the contents can be used up to the stated expiry date when stored at 15-25 °C.

The bottles must always be kept tightly closed.

Additional instructions

For professional use only.
The application must be carried out by qualified personnel only.

National guidelines for work safety and quality assurance must be followed.

Microscopes equipped according to the standards must be used.

Protection against infection

Effective measures must be taken to protect against infection in line with laboratory guidelines.

Instructions for disposal

The package must be disposed of in accordance with the current disposal guidelines. Used solutions and solutions that are past their shelf-life must be disposed of as special waste in accordance with local guidelines.

Auxiliary reagents

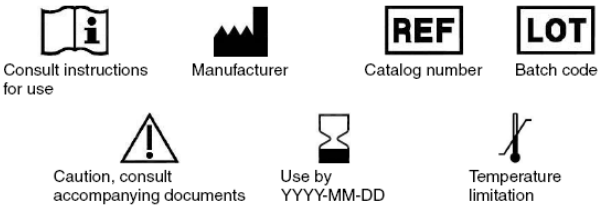
Cat. No. 64969	Harleco® Krystalon™ Mounting Medium	50 mL, 500 mL
Cat. No. 104699	Immersion oil for microscopy	100 mL, 500 mL
Cat. No. 1217	Buffer, Phosphate pH 6.4	1 L, 4 L
Cat. No. 1218	Buffer, Phosphate, pH 6.8	1 L, 4 L
Cat. No. 1219	Buffer, Phosphate pH 7.0	1 L, 4 L
Cat. No. 6442	Water, Deionized Distilled, ASTM Type II	4 L, 20 L
Cat. No. 65044A	HemaColor® Stain Solution (fixative)	4 L

Hazard classification

Cat. No. 619 Please observe the hazard classification printed on the label and the information given in the safety data sheet. The safety data sheet is available on the website and on request.

Literature

1. Löffler, H., Rastetter, J., Haferlach, T, Atlas der klinischen Hämatologie, 2004, Springer-Verlag Berlin Heidelberg
2. Routine Cytological Staining Techniques: Theoretical Background and Practice, Mathilde E. Boon and Johanna S. Drijver, 1986, Elsevier Science Publishing Company
3. Conn's Biological Stains: A Handbook of Dyes, Stains and Fluorochromes for Use in Biology and Medicine, 10th Edition, (ed. Horobin, R.W., and Kiernan, J.A.) Bios, 2002



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