BIOGNOST®

WRIGHT-GIEMSA, SOLUTION FOR REPTILES

IVD In vitro diagnostic medical device CE

Modified Romanowsky dye for staining in reptile hematology

INSTRUCTIONS FOR USE

REF Catalog number: WRGG-OT-100 (100 mL) WRGG-OT-500 (500 mL) WRGG-OT-1L (1000 mL) WRGG-OT-2.5L

Introduction

In hematology polychromatic Romanowsky dyes are a standard for blood smears and bone marrow staining. Various sorts of Romanowsky dyes (Giemsa, May-Gruenwald, Leishman, Wright, Wright-Giemsa, Jenner) contain different ratios of methylene bluing reagent used as the cation component (and the reagent-related thiazine dyes, such as azure B) and eosin Y as the anion component. Cation and anion components interaction creates a well known Romanowsky effect that cannot be achieved if each component is being used individually. Purple color indicates the effect's presence. Staining intensity depends on the azure B content, as well as azure B to eosin Y ratio, while a few other factors affect the result of staining: working solution pH value and buffer solution, fixation method and dye exposure time. BioGnost's Wright-Giemsa, solution for reptiles is used for differentiating nuclear and/or cytoplasmatic morphology of thrombocytes, erythrocytes, and lymphocytes in blood smear of reptiles.

Product description

- **WRIGHT-GIEMSA, SOLUTION FOR REPTILES** – Modified Romanowsky dyefor staining in reptile hematology

Other slides and reagents that may be used in staining:

- Glass slides used in hematology, such as VitroGnost STANDARD GRADE or high quality glass slides used in histopathology and cytology, such as VitroGnost SUPER GRADE or one of more than 30 models of VitroGnost glass slides
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm
- BioGnost's immersion media, such as Immersion oil, Immersion oil, types A, C, FF, 37, or Immersion oil Tropical Grade
- BioGnost's Buffer tablets, pH 6.8 or 7.2

Preparation of solutions

Buffer solution, pH 6.8

- Dissolve 1 pH 6.8 buffer tablet in 1 liter of distilled water while stirring.
  Note: During the staining process it is possible to use pH 7.2 buffer solution or a combination of pH 6.8 and 7.2 buffer solutions. The process's results can differentiate in shift toward red or blue on the color spectrum.

Diluted Wright-Giemsa, solution for reptiles for A2) Procedure of staining blood smear in staining jar

- After step 2, dilute used Wright-Giemsa, solution for reptiles using Buffer solution pH 6.8 in 1:1 ratio (for instance, 50 mL of Wright-Giemsa, solution for reptiles and 50 mL of Buffer solution pH 6.8.

A1) Procedure of staining blood smear on a staining rack

1.	Let the smear dry	
2.	Apply Wright-Giemsa, solution for reptiles (1 mL) to the smear and let it react	3 min
3.	Apply Buffer solution pH 6.8 (1 mL) to Wright-Giemsa, solution for reptiles and gently stir; let it react	6 min
	Note: After staining tilt the preparation and decant the solution.	
4.	Add Buffer solution pH 6.8, gently stir and let it react	1 min
5.	Rinse well in Buffer solution pH 6.8 until the excessive dye is removed.	
6.	Dry the slide	

A1) Procedure of staining blood smear in a cuvette

1.	Let the smear dry	
2.	Immerse the section into Wright-Giemsa solution, solution for reptiles	3 min
3.	Immerse the section into **diluted** Wright-Giemsa solution, solution for reptiles	6 min
4.	Immerse the section in Buffer solution pH 6.8 and let it react	1 min
5.	Rinse well in Buffer solution pH 6.8 until the excessive dye is removed	
6.	Dry the preparation	

Result (pH 6.8)

Nucleus - purple
Lymphocyte cytoplasm - blue
Monocyte cytoplasm - grey-blue
Neutrophil granule - light purple
Eosinophil granule - red-orange
Basophil granule - dark purple to black
Thrombocytes - purple
Erythrocytes - reddish

Note

Time periods of staining processes are not entirely standardized in clinical and laboratory practical experience. Time periods specified in the instruction approximately correspond to a longtime work practice with optimal results. Intensity of staining depends on the period of immersion in the dye. Real staining protocol depends on personal requests and priorities.

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. Reagents 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 Wright-Glemsa, solution for reptiles in a tightly closed original package at temperature between 15°C and 25°C. Do not keep in cold 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. Beck, R.C. (1938): *Laboratory Manual of Heamtological Technique,* Philadelphia, W.B. Saunders & Co.
2. Dacie, J. et Lewis S. (1995): *Practical haematology*, 4th ed., London, Churchill Livingstone.
3. Garcia, L. S. (2001): *Diagnostic Medical Parasitology*, 4th ed., Washington, D.C., ASM Press.
4. Giemsa, G. (1922): Das Wesen der Giemsa-Farbung, *Zentralb f Bakt*; 89, p 99-106.
5. Kiernan, J.A. (2008): *Histological and histochemical methods: Theory and Practice,* 4th ed., Bloxham, Scion Publishing Ltd.
6. May, R. et Grünwald L. (1909): *Über die Farbung von Feutchpraparaten mit meiner Azur-Eosine methode,* Deutsche med Xschr, 35, p 1751-1752.

WRGG-OT-X, V4-EN3, 29 September 2017, AK/VR

Refer to the supplied documentation	Storage temperature range	Number of tests in package	REF	Product code	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					