BIOGNOST®

WRIGHT'S SOLUTION

IVD *In vitro* diagnostic medical device CE

Polychromatic solution of eosin, Methylene Blue and azure dyes
For staining in hematology, cytology and cytogenetics

INSTRUCTIONS FOR USE

REF Product code: WR-OT-100 (100 mL) WR-OT-500 (500 mL) WR-OT-1L (1000 mL)

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, 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's solution is used for differentiating nuclear and/or cytoplasmatic morphology of thrombocytes, erythrocytes, and lymphocytes in blood smear or bone marrow aspirates. Besides in hematology, it is also used for staining chromosomes in cytogenetics for detecting chromosomal aberrations and diagnosing genetic syndromes.

Product description

- **WRIGHT'S SOLUTION** - Solution of eosin, Methylene Blue and azure dyes in methanol with added stabilizer.

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 is used for hematology and cytology smears.
Buffer solution pH 7.2 is used for staining hematology smears expected to contain blood parasites.

- Dissolve 1 buffer tablet in 1 liter of distilled water while stirring. Filter after dissolving.
  Note: During the staining process it is possible to use pH 6.8 or 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.

Wright's working solution for vertical staining

- Combine 30mL of Wright's solution with 150 ml of distilled or demineralized water and with 20 ml of pH 6.8 or pH 7.2 buffer solution. Let it set for 10 min. Filter before use.

Working Wright solution for staining in automatic stainer

- Combine 50mL of Wright's solution with 220 ml of distilled or demineralized water and with 30 ml of pH 6.8 or pH 7.2 buffer solution. Let it set for 10 min. Filter before use.

A1) Procedure of horizontal smear staining (on a staining rack)

1.	Dry the preparation	
2.	Place the section in the horizontal position and cover it with 1 ml of **undiluted Wright's solution**	1 min
3.	Add 1 mL of **Buffer solution** pH 6.8 or pH 7.2 , gently stir and let it react	4 min
4.	Rinse with **Buffer solution,** pH 6.8 or pH 7.2 through two exchanges	2 exchanges, 1 minute each
5.	Dry the preparation	

A1) Procedure of vertical smear staining (in a cuvette)

1.	Dry the preparation	
2.	Immerse the section into **non-diluted Wright's solution**	3 min
3.	Immerse the section into the **Wright's working solution for vertical staining**	6 min
4.	Rinse with **Buffer solution,** pH 6.8 or pH 7.2 through two exchanges	2 exchanges, 1 minute each
5.	Dry the preparation	

A3) Procedure of staining smear in automatic stainer

1.	Dry the preparation	
2.	Immerse the section into **non-diluted Wright's solution**	3 min
3.	Immerse the section into the **Wright's working solution for staining in automatic stainer**	6 min
4.	Rinse with **Buffer solution,** pH 6.8 or pH 7.2	1 min
5.	Rinse in tap water	2 min
6.	Dry the preparation	

	Result (pH 6.8)	Result (pH 7.2)
nucleus	red-purple	red-purple
lymphocyte cytoplasm	blue	blue
monocyte cytoplasm	grey-blue	grey-blue
neutrophil granules	bright purple	light purple
eosinophil granules	dark red to red-brown	brick red to red-brown
basophil granules	dark purple to black	dark purple to black
thrombocytes	purple	purple
erythrocytes	reddish	reddish-grey

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's solution 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.

WR-OT-X, V9-EN6, 21.05.2019., IŠP/IVR

	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	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						