BIOGNOST®

BIO-DIFF KIT

IVD In vitro diagnostic medical device CE

Three reagent kit for staining in hematology

Contains a fixative and a red and blue component for fast and effective hematological samples staining

INSTRUCTIONS FOR USE

REF Catalog number: BD-K-100 (3 x 100 mL) BD-K-500 (3 x 500 mL) BD-K-1L (3 x 1000 mL) BD-K-2.5L (3 x 2500 mL)

Introduction

Polychromatic Romanowsky dyes are a standard in staining blood smears and bone marrow in hematology. Various sorts of Romanowsky dyes (Giemsa, May-Gruenwald, Leishman, Wright, Jenner and others) contain different ratios of methylene blue used as cation component (and the reagent-related thiazine dyes, such as azure B) and eosin Y as anion component. Cation and anion components interaction creates a well known Romanowsky effect that cannot be achieved if each component is being used individually; color purple 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 and buffer solution pH value, buffer substance type, fixing method, and dye exposure time. BioGnost's Bio-Diff kit stains hematological preparations in a short period of time and provides precise staining results, such as results of May-Gruenwald Giemsa method. Each part of the set is stabilized separately and prepared according to the highest standards. Besides Bio-Diff reagents, the kit also contains buffer tablets with 6.8 and 7.2 pH values.

Product description

- **BIO-DIFF KIT** – Fast and efficient hematological samples staining kit.

Kit contains:	BD-K-100 (3 x 100 mL)	BD-K-500 (3 x 500 mL)	BD-K-1L (3 x 1000 mL)	BD-K-2.5L (3 x 2500 mL)
Bio-Diff 1 reagent	100 mL (BD1-OT-100)	500 mL (BD1-OT-500)	1000 mL (BD1-OT-1L)	2500 mL (BD1-OT-100)
Bio-Diff 2 reagent	100 mL (BD2-OT-100)	500 mL (BD2-OT-500)	1000 mL (BD2-OT-1L)	2500 mL (BD2-OT-100)
Bio-Diff 3 reagent	100 mL (BD3-OT-100)	500 mL (BD3-OT-500)	1000 mL (BD3-OT-1L)	2500 mL (BD3-OT-100)
Buffer tablet pH 6.8	2 pcs	5 pcs	10 pcs	15 pcs
Buffer tablet pH 7.2	2 pcs	5 pcs	10 pcs	15 pcs

Other sections and reagents that may be used in staining:

- BioGnost's immersion oils, such as BioGnost's Immersion oil, Immersion oils types C, A, FF, 37 and Tropical Grade
- 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

Preparation of solutions

Buffer solution, pH 6.8 or 7.2

- Dissolve 1 pH 6.8 buffer tablet in 1 liter of distilled water while stirring. Filter the solution.

Blood smear/bone marrow sample staining procedure

1.	Let the smear dry	
	Note: Prepare a freshly samples peripheral blood smear	
2.	Dip the section into Bio-Diff 1 reagent	5 x 1 second
3.	Decant the excessive reagent from the section onto filter paper	
4.	Dip the section into Bio-Diff 2 reagent	3 x 1 second
	Note: extend the incubation period if a stronger hue of red/purple is required	up to 5 x 1 second
5.	Decant the excessive reagent from the section onto filter paper	
6.	Dip the section into Bio-Diff 3 reagent	6 x 1 second
	Note: decrease the incubation period if a stronger hue of red/purple is required	5 x 1 second
7.	Rinse the section in pH 6.8 buffer solution	1 min (with agitation)
8.	Dry the preparation	

Parasitology *(Leishmania, Toxoplasma, Microsporadia)* and microbiology samples *(Cryptosporidium, Pneumocystis carinii)* staining procedure

1.	Dip the section into Bio-Diff 1 reagent	1 min
2.	Decant the excessive reagent from the section onto filter paper	
3.	Dip the section into Bio-Diff 2 reagent	25 seconds
4.	Decant the excessive reagent from the section onto filter paper	
5.	Dip the section into Bio-Diff 3 reagent	25 seconds
6.	Rinse the section in pH 7.2 buffer solution	1 min (with agitation)
7.	Dry the slide	

Sperm staining procedure

Preparing the sperm smear: Add 15 μL of fresh sperm sample on one side of the glass slide and create a thin and homogeneous smear. Let the smear dry (10 minutes).

1.	Dip the section into Bio-Diff 1 reagent	5 x 1 second
2.	Decant the excessive reagent from the section onto filter paper	
3.	Dip the section into Bio-Diff 2 reagent	5 x 1 second
4.	Decant the excessive reagent from the section onto filter paper	
5.	Dip the section into Bio-Diff 3 reagent	5 x 1 second
6.	Rinse the section in pH 7.2 buffer solution	1 min (with agitation)
7.	Dry the slide	

In order to create a permanent sample, apply appropriate type of DPX medium on both stained and dried section (BioMount DPX medium for covering/mounting cover slides). Cover the section with VitroGnost cover glass.

Result

Head - homogeneous dark purple
Acrosome - light purple
Mid piece and tail - dark purple
Background - light pink

Histology sections staining procedure

a) preparation of histology sample

- Fix the sample (Formaldehyde NB 4%, Formaldehyde NB 10%), 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; using xylene (BioClear) or 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 mount them on a VitroGnost Super Grade glass slide.

b) staining histology sample

Note: do not use Bio-Diff 1 reagent (used as fixative for non-histology samples)

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.	Dip the section into Bio-Diff 2 reagent and gently stir	7 seconds
6.	Dip the section into Bio-Diff 3 reagent and gently stir	5 seconds
7.	Rinse the smear using Buffer solution pH 7.2	1 min (with agitation)
8.	Decant the excessive reagent from the section onto filter paper	
9.	Dehydrate and differentiate in 95% alcohol (Histanol 95) while gently stirring	10 seconds
10.	Dehydrate the section by using 100% alcohol (Histanol 100)	1 min
11.	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 VitroGnost cover glass.

Cytobacteriology samples staining procedure (urine, punctates, CSF)

1.	Let the cytology smear dry	
2.	Dip the section into Bio-Diff 1 reagent	5 seconds
	Note: Incubate CSF for a longer period of time	1 min
3.	Decant the excessive reagent from the section onto filter paper	
4.	Dip the section into Bio-Diff 2 reagent	3 x 1 seconds (CSF 2 x 1 second)
	Note: extend the incubation period if a stronger hue of red/purple is required	do 5 x 1 second
5.	Decant the excessive reagent from the section onto filter paper	
6.	Dip the section into Bio-Diff 3 reagent	6 x 1 seconds (CSF 2 x 1 second)
	Note: decrease the incubation period if a stronger hue of red/purple is required	5 x 1 second
7.	Rinse the section in pH 7.2 buffer solution	1 min (with agitation)
8.	Dry the preparation	

Results (blood smear)

Nuclei - red to purple
Lymphocytes - plasma is colored blue
Monocytes - plasma is colored grey-blue
Neutrophil granulocytes - light purple
Eosinophil granulocytes - bright red to red-brown
Basophil granulocytes - dark purple to black
Thrombocytes - purple
Erythrocytes - reddish
Blood parasites - red (nuclei), blue (cytoplasm)

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 and application. 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 (available on request).

Storing, stability and expiry date

Keep Bio-Diff kit 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. Giemsa, G. (1922): Das Wesen der Giemsa-Farbung, *Zentralb f Bakt*; 89, p 99-106.
4. Kiernan, J.A. (2008): *Histological and histochemical methods: Theory and Practice,* 4th ed., Bloxham, Scion Publishing Ltd.
5. May, R. et Grünwald L. (1909): *Über die Farbung von Feutchpraparaten mit meiner Azur-Eosine methode,* Deutsche med Xschr, 35, p 1751-1752.

BD-X, V13-EN9, 26 August 2021, 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	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						