BIOGNOST®

BIO-DIFF RTU KIT

IVD In vitro diagnostic medical device CE

Three-reagent kit for rapid Ready-to-Use staining

Contains a fixative and a red and blue component for rapid and effective staining

INSTRUCTIONS FOR USE

REF Catalog number: BD-RTU-100 (3 x 100 mL)

Introduction

BioGnost's Bio-Diff RTU kit enables rapid, simple and high quality staining according to May-Gruenwald-Giemsa staining method. Except for standard staining of blood smears, the kit may be used for staining parasites and fungae, histology samples embedded in paraffin, and cytology smears. Advantages of Bio-Diff RTU kit: extremely rapid staining **(14 seconds)** of blood and cytology smears; practical and simple for use owing to **impermeable polypropylene** jars filled with 100 ml of reagent that enable direct dipping of sections (for **100-200** tests); **buffered solutions** that enable consistent quality in staining each section. Each part of the set is stabilized separately and prepared according to the highest standards.

Product description

BIO-DIFF RTU KIT – Kit for rapid and efficient staining of hematology, cytology, histology, parasitology, and mycology samples.

The kit contains:	100-200 tests (BD-RTU-100)
Bio-Diff 1 RTU reagent	100 mL (BD1-RTU)
Bio-Diff 2 RTU reagent	100 mL (BD2-RTU)
Bio-Diff 3 RTU reagent	100 mL (BD3-RTU)
Buffer tablet, pH 6.8	2 pcs
Buffer tablet, pH 7.2	2 pcs

Preparation of solutions

Buffer solution (pH 6.8 or 7.2)

Dissolve 1 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 the peripheral blood smear by draining blood from a fresh blood sample	
2.	Dip the section into Bio-Diff 1 RTU reagent	5 x 1 second
3.	Decant the excessive reagent from the section onto filter paper	
4.	Dip the section into Bio-Diff 2 RTU reagent	3 x 1 second
	Note: extend the incubation period if a stronger hue of red/purple is required	5 x 1 second
5.	Decant the excessive reagent from the section onto filter paper	
6.	Dip the section into Bio-Diff 3 RTU 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 slide	

Staining method of parasitology (*Leishmania, Toxoplasma, Microsporadia*) and microbiology samples (*Cryptosporidium, Pneumocystis carinii*)

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

Sperm staining procedure

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

1.	Dip the section into Bio-Diff 1 RTU reagent	5 x 1 second
2.	Decant the excessive reagent from the section onto filter paper	
3.	Dip the section into Bio-Diff 2 RTU reagent	5 x 1 second
4.	Decant the excessive reagent from the section onto filter paper	
5.	Dip the section into Bio-Diff 3 RTU 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

Histological 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; in xylene (BioClear) or in a 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

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 RTU reagent and gently stir	7 seconds
6.	Dip the section into Bio-Diff 3 RTU 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 RTU 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 RTU 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 RTU 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.

Storing, stability and expiry date

Keep Sperm-Diff RTU 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-RTU-100, V8-EN7, 2 May 2018, AK/VR

Refer to the supplied documentation	Storage temperature range	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						