

# BIO-DIFF 2 REAGENT

IVD *In vitro* diagnostic medical device



## Red component of Bio-Diff kit

### INSTRUCTIONS FOR USE

REF Product code: BD2-OT-100 (100 mL)      BD2-OT-500 (500 mL)      BD2-OT-1L (1000 mL)      BD2-OT-2.5L (2500 mL)

#### Introduction

Bio-Diff 2 reagent is a component of BioGnost's Bio-Diff kit. Contains eosin Y dye in phosphate buffer. Other reagents found in the kit is Bio-Diff 1 reagent (red component), Bio-Diff 3 reagent (blue component), and pH 6.8 and 7.2 buffer tablets. Bio-Diff 1 reagent contains methyl alcohol, while Bio-Diff 3 contains azure dyes. Red and blue 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. BioGnost's Bio-Diff kit efficiently stains hematology and cytology sections in a short period of time and provides precise staining results, such as results of the May-Gruenwald-Giemsa method. Each part of Bio-Diff kit is stabilized separately and prepared according to the highest standards.

#### Product description

**BIO-DIFF 2 REAGENT** – eosin Y dye solution in phosphate buffer.

#### Using Bio-Diff 2 reagent as a component of Bio-Diff kit

##### Other slides and reagents that may be used in staining:

- BioGnost's immersion oils, such as BioGnost's Immersion oil, Cedarwood oil, Immersion oils types C, A, FF 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
- Other components of Bio-Diff kit: Bio-Diff 1 reagent (catalog number BD1-OT-100, BD1-OT-500, BD1-OT-1L, BD1-2.5L) and Bio-Diff 3 reagent (product code BD3-OT-100, BD3-OT-500, BD3-OT-1L, BD3-OT-2.5L) and Buffer tablets pH 7.2 (product code PT-72-50, PT-72-100) or Buffer tablets pH 6.8 (catalog number PT-68-50, PT-68-100)

#### 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 the peripheral blood smear by draining blood from a fresh blood sample	
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 Note: extend the incubation period if a stronger hue of red/purple is required	3 x 1 second 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 Note: decrease the incubation period if a stronger hue of red/purple is required	6 x 1 second 5 x 1 second
7.	Rinse the section in pH 6.8 buffer solution	1 min (with agitation)
8.	Dry the preparation	

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

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 (at least 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

## 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  $\mu\text{m}$  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 fixation agent 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.

### Storing, stability and expiry date

Keep Bio-Diff 2 reagent 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 Hematological Technique*, Philadelphia, W.B. Saunders & Co.
2. Dacie, J. et Lewis S. (1995): *Practical haematology*, 4<sup>th</sup> ed., London, Churchill Livingstone.
3. Giemsa, G. (1922): Das Wesen der Giemsa-Färbung, *Zentralbl f Bakt*; 89, p 99-106.
4. Kiernan, J.A. (2008): *Histological and histochemical methods: Theory and Practice*, 4<sup>th</sup> ed., Bloxham, Scion Publishing Ltd.
5. May, R. et Grünwald L. (1909): *Über die Färbung von Feuchtpreparaten mit meiner Azur-Eosine methode*, Deutsche med Xschr, 35, p 1751-1752.

BD2-X, V1-EN1, 24 July 2020, KB/ISP

	Obavezno proučiti priloženu dokumentaciju		Temperaturni raspon čuvanja		Broj testova u pakovanju		Kataloški broj		Europska sukladnost
	Pročitati priloženu uputu		Čuvati od topline i sunčevog svjetla		Vrijedi do		Broj serije		Proizvođač
	Samo za <i>in vitro</i> dijagnostičku primjenu		Čuvati na suhom		Opazuj lomljivo				

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