

BIO-DIFF 1 RTU REAGENT



IVD *In vitro* diagnostic medical device

Classified acc. to Regulation (EU) 2017/746 - **Class A** device

Fixative component of Bio-Diff RTU kit (rapid Ready-to-Use staining)

INSTRUCTIONS FOR USE

BASIC UDI number	385889212HPC3010302HMCA		
EMDN code	W0103010302		
REF	Catalogue number	Volume	UDI-DI number
BD1-RTU		100 mL	03858890003154



Intended use and test principle

Bio-Diff 1 reagent RTU is a fixative component of BioGnost's Bio-Diff RTU kit. It contains methyl alcohol whose primary role is fixing smears and the first step in staining smears using Bio-Diff RTU kit. Other reagents found in the kit are Bio-Diff 2 RTU reagent (red component), Bio-Diff 3 RTU reagent (blue component), and pH 6.8 and 7.2 buffer tablets. Reagents are filled in jars that may be used as staining jars. Bio-Diff 2 RTU contains eosin Y dye, while Bio-Diff 3 RTU 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 RTU kit efficiently stains hematology and cytology smears in a short period of time (precisely **14 seconds**), as well as histology, parasitology and micology samples, and provides precise staining results, such as results of the May-Gruenwald-Giemsa method. Each component of Bio-Diff RTU kit is stabilized separately and prepared according to the highest standards.

Product description

- **BIO-DIFF 1 RTU REAGENT** – Alcoholic fixative component of Bio-Diff RTU kit

Using Bio-Diff 1 RTU reagent as a component of Bio-Diff RTU kit

Additional reagents and materials that can be used in staining:

- BioGnost's immersion oils, such as BioGnost's Immersion oil, Immersion oils types C, A, FF and Tropical Grade
- VitroGnost slides and coverslips for use in histopathology and cytology
- Other components of Bio-Diff RTU kit: Bio-Diff 2 RTU reagent (catalog number BD2-RTU) and Bio-Diff 3 RTU reagent (catalog number BD3-RTU,) and Buffer tablets pH 7.2 (catalog number 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 smear into Bio-Diff 1 RTU reagent	5 x 1 second
3.	Decant the excessive reagent from the smear onto filter paper	
4.	Dip the smear into Bio-Diff 2 RTU 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 smear onto filter paper	
6.	Dip the smear into Bio-Diff 3 RTU 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 smear in pH 6.8 buffer solution	1 min (with agitation)
8.	Dry the smear	

Staining method for parasitology (*Leishmania*, *Toxoplasma*, *Microsporidia*) and microbiology samples (*Cryptosporidium*, *Pneumocystis carinii*)

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

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 smear into Bio-Diff 1 RTU reagent	5 x 1 second
2.	Decant the excessive reagent from the smear onto filter paper	
3.	Dip the smear into Bio-Diff 2 RTU reagent	5 x 1 second
4.	Decant the excessive reagent from the smear onto filter paper	
5.	Dip the smear into Bio-Diff 3 RTU reagent	5 x 1 second
6.	Rinse the smear 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 smear (BioMount DPX medium for covering/mounting cover slides). Cover the smear with VitroGnost cover glass.

Result

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

Cytobacteriology samples staining procedure (urine, punctates, CSF)

1.	Let the cytology smear dry	
2.	Dip the smear 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 smear onto filter paper	
4.	Dip the smear 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 smear onto filter paper	
6.	Dip the smear 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 smear in pH 7.2 buffer solution	1 min (with agitation)
8.	Dry the smear	

Results for staining cytological samples

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)

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the staining procedure described in this Instruction for use may cause differences in staining results.

Sample preparation and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples using modern technology and mark them clearly. Be sure to follow the manufacturer's handling instructions. To avoid errors, staining, mounting of the slides, and diagnosis can only be carried out by qualified personnel. Use a microscope equipped according to medical diagnostic laboratory standards.

If a serious incident occurs during use of this product or as a result of its use, please report it to the manufacturer or authorized representative and competent authority.

Safety at work and environmental protection

Handle the product in accordance with occupational health and environmental protection guidelines. Used and expired solutions must be disposed of as special waste following national guidelines. Reagents used in this procedure can pose a danger to human health. The examined tissue samples are potentially infectious, and it is necessary to take the measures needed to protect human health in accordance with the guidelines of good laboratory practice. It is mandatory to read and act according to the information and warning signs printed on the product label and in the Safety Data Sheet, which is available on request.

Storage, stability, and shelf life

Upon receipt, store the product in a dry place and well-closed original packaging at a temperature of +15 °C to +25 °C. Do not freeze or expose to direct sunlight. After first opening, the product can be used until the specified expiry date, if stored properly. The expiration date is printed on the product label.

References

- Beck, R.C. (1938): *Laboratory Manual of Hematological Technique*, Philadelphia, W.B. Saunders & Co.
- Dacie, J. et Lewis S. (1995): *Practical haematology*, 4th ed., London, Churchill Livingstone.
- Giemsa, G. (1922): Das Wesen der Giemsa-Färbung, *Zentralb f Bakt*; 89, p 99-106.
- Kieman, J.A. (2008): *Histological and histochemical methods: Theory and Practice*, 4th ed., Bloxham, Scion Publishing Ltd.
- May, R. et Grünwald L. (1909): Über die Färbung von Feuchtpreparaten mit meiner Azur-Eosine methode, *Deutsche med Xschr*, 35, p 1751-1752.

Warnings and precautions regarding the materials contained in the product:

	H225	Highly flammable liquid and vapour.
	H301+H311+H331	Toxic if swallowed, in contact with skin or if inhaled.
	H370	Causes damage to organs (eyes).
	P210	Keep away from heat, hot surfaces, sparks, open flames and other sources of ignition. No smoking.
	P233	Keep container tightly closed.
	P280	Wear protective gloves/protective clothing/eye protection/face protection.
	P301+P310	IF SWALLOWED: Immediately call a POISON CENTER/doctor.
	P302+P352	IF ON SKIN: Wash with plenty of soap and water.
	P304+P340	IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing.
	P308+P311	IF exposed or concerned: Call a POISON CENTER or doctor.

BD1-RTU-IFU, EN2, 03 November, 2025, IŠP

	Manufacturer		Batch code		Consult instructions for use		European conformity
	Date of manufacture		Catalogue number		Caution		Unique device identifier
	Use-by date		Temperature limit		In vitro diagnostic medical device		

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Version	Description / reason for change	Date
2.	Addition of: the QR code, table with the Warnings and precautions, revised table with the symbols	03.11.2025.