BIOGNOST®

BIO-DIFF RTU KIT

CE IVD *In vitro* diagnostic medical device Classified acc. to Regulation (EU) 2017/746 - **Class A** device

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

Contains a fixative, a red and a blue component for rapid polychromic staining

INSTRUCTIONS FOR USE

BASIC UDI number	385889212HPC3010302HMCA	
EMDN code	W0103010302	
REF **Catalogue number**	**Volume**	**UDI-DI number**
BD-RTU-100	3x100 mL	03858890003147

Intended use and test principle

BioGnost's Bio-Diff RTU kit enables rapid, simple and high-quality polychromic 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, cytology smears, and for the detection of *Helicobacter pylori* on histological sections. 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:	**3 x 100 mL (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/demineralized 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	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 smear onto filter paper	
6.	Dip the smear 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 smear in pH 6.8 buffer solution	1 min (with agitation)
8.	Dry the smear	

Staining method of parasitology (*Leishmania, Toxoplasma, Microsporadia*) 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	

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	

Histological sections staining procedure

a) preparation of histological sections for staining

- Fix (Formaldehyde NB 4%, Formaldehyde NB 10%) and process the tissue sample
- Embed the tissue in a paraffin block (BioWax 52/54, BioWax 56/58, BioWax Plus 56/58, BioWax Blue)

- Cut the paraffin block into 4-6 μm thin slices and mount on a VitroGnost microscope slide

b) sample staining procedure

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 covering/mounting medium. If BioClear xylene was used, use one of BioGnost's xylene-based mountants (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate mountant is BioMount New. Cover the section with a VitroGnost cover glass.

Results for staining of cytological and histological 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)
Helicobacter pylori – dark blue
Tissue cellular elements – blue to pink

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 smear	

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.

Results for sperm staining

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

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.

Literature

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.

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.

BD-RTU-IFU_EN8, 30 September 2025, IŠP

	Manufacturer	LOT	Batch code		Consult instructions for use	CE	European conformity
	Date of manufacture	REF	Catalogue number		Caution	UDI	Unique device identifier
	Use-by date	°C	Temperature limit	IVD	*In vitro* diagnostic medical device		

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Version	Description / reason for change	Date
8.	Addition of: the QR code, table with the Warnings and precautions, revised table with the symbols, staining results of the Helicobacter pylori	30.09.2025.