BUFFER SOLUTION 6.8

IVD *In vitro* diagnostic medical device CE

Buffer solution for use in hematology, 6 mM

INSTRUCTIONS FOR USE

REF Product code: PUF68-0T-1L (1000 mL) PUF68-0T-2.5L (2500 mL) PUF68-0T-5L (5000 mL)

Introduction

BioGnost's Buffer solution pH 6.8 contains sodium and potassium phosphates that enable the solution to be buffered at pH 6.8. The buffer solution's molality is 6 nM and it is recommended for use in practical hematology during blood smears staining procedures. In that case the buffer solution is essential for preparation of diluted Giemsa/May-Gruenwald/Wright/Leishman solutions and for rinsing stained samples without causing destaining of stained cells. Buffer solutions are solutions of weak acids and theirs salts or weak bases and their salts. They enable maintaining approximately constant pH value by adding a certain amount of strong acids or bases.

Product description

- **BUFFER SOLUTION pH 6.8** – Phosphate buffer solution, molality 6 mM.

Example of Buffer solution pH 6.8 use with hematology staining:

Preparation of solutions

Working Giemsa solution for standard staining method

Add 10mL of Giemsa solution to 190 ml of pH 6.8 buffer solution, stir well and let it sit for 10 min. Filter if necessary.

Working Giemsa solution for perioperative staining method

Add 10mL of Giemsa solution to 50 ml of pH 6.8 buffer solution, stir well and let it sit for 10 min. Filter if necessary.

A1) Blood smear staining procedure using Giemsa solution

- Prepare the peripheral blood smear by draining blood from a fresh blood sample
- Fixate the previously dried blood smears by immersing them for 5 min in methanol (Histanol M)
- Immerse the fixated smear in the working Giemsa solution for 15-20 min
- Rinse the smear twice in the pH 6.8 buffer solution during 1 min time
- Dry the preparation

Result (pH 6.8)

Nucleus - red to purple
Lymphocyte plasma - blue
Monocyte plasma - grey-blue
Neutrophil granule - light purple
Eosinophil granule - red to grey-blue
Basophil granule - dark purple
Thrombocytes - purple
Erythrocytes - reddish
Blood parasites - red nuclei

A2) Blood smear staining procedure using May-Gruenwald Giemsa (Pappenheim) standard method

- Prepare the peripheral blood smear by draining blood from a fresh blood sample.
- Let the smear dry.
- Apply May-Gruenwald solution to the dried smear and let it be active for 3-5 mins.
- Rinse the smear shortly in pH 6.8 buffer solution.
- Apply the Giemsa solution to the dried smear and let it be active for 15-20 min.
- Rinse the smear shortly in pH 6.8 buffer solution.
  Note: If necessary, apply a smaller volume of the buffer solution on the slide in order to thoroughly remove the excessive dye and to make the stained structures clearly visible. Rinse the solution after 10-30 seconds.
- Rinse the smear shortly in pH 6.8 buffer solution.

A3) Blood smear staining procedure using May-Gruenwald Giemsa (Pappenheim) method

- Prepare the peripheral blood smear by draining blood from a fresh blood sample.
- Let the smear dry.
- Apply the May-Gruenwald solution to the dried smear and let it be active for 1-2 mins.
- Rinse the smear shortly in pH 6.8 buffer solution.
- Apply the Giemsa solution to the dried smear and let it be active for 5 min.
- Rinse the smear shortly in pH 6.8 buffer solution.
  Note: If necessary, apply a smaller volume of the buffer solution on the slide in order to thoroughly remove the excessive dye and to make the stained structures clearly visible. Rinse the solution after 10-30 seconds.
- Dry the slide.

Result (pH 6.8)

Nucleus - purple
Lymphocyte plasma - blue
Monocyte plasma - grey-blue
Neutrophil granule - light purple

Eosinophil granule - red to dark purple
Basophil granule - dark purple to black
Thrombocytes - purple
Erythrocytes - reddish

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. 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. In order to avoid an erroneous result, a positive and negative check is advised before application.

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 Buffer solution pH 6.8 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. Fritsch, E. F., Maniatis, T. et Sambrook, J. (1989): Molecular Cloning: A Laboratory Manual, 2nd ed., New York, Cold Spring Harbour Laboratory Press.
2. Ionatamishvili, T. V. et al. (1970): Tablets for adjusting and checking pH meters, *Measurement techniques,* 14 (2): p 310-312.
3. Robinson, R. A. et Stokes, R. A. (1968): Electrolyte solutions, 2nd ed., London, Butterworths.

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	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
	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						