

# EA 65 REAGENT, PAP 3D

IVD In vitro diagnostic medical device



## Cytoplasmic staining reagent acc. to Papanicolaou - blue-green hue Polychromatic counterstain for gynecological and non-gynecological samples in cytology

### INSTRUCTIONS FOR USE

REF Catalogue number: EA65D-OT-100 (100 mL) EA65D-OT-500 (500 mL) EA65D-OT-1L (1000 mL) EA65D-OT-2.5L (2500 mL)

#### Introduction

EA 65 reagent, Pap 3D reagent is an alcoholic solution of two acid dyes, Eosin Y and Light Green SF, with added phosphotungstic acid (PTA). The first step in using the Papanicolaou staining method implies nuclear staining with a hematoxylin solution, and next two steps consist of counterstaining using the monochromatic OG-6 or Orange II reagents and one of the polychromatic EA reagent formulations. The Orange G and Orange II molecules stain the cytoplasm, and in later stages of the procedure it remains only in the mature, keratinized cells. One of the polychromatic EA solutions is used in the third step. Those solutions differentiate between squamous cells. Test samples can be gynecological and non-gynecological, such as sputum, urine, diarrhea, and cytological puncture samples. In order to obtain optimal staining results, EA 65 reagent, Pap 3D has properties completely in compliance with other BioGnost's reagents for cytological smearing acc. to Papanicolaou - Hematoxylin HP reagent, Pap 1A or 1B, OG-6 reagent, Pap 2A, and Orange G II reagent, Pap 2B.

#### Product description

**EA 65 REAGENT, PAP 3D** - Polychromatic counterstain for staining non-gynecological samples in cytology Contains BSC certified dyes Eosin Y, Light Green SF and Bismarck Brown with phosphotungstic acid that participates in selective staining of cytoplasm of hormonally different cells.

#### Preparing the cytological smear for staining

There are two methods of collecting and preparing the cytological samples:

1. After collecting the cytological sample, place it on the microscope slide (VitroGnost), fixate it immediately with a fixative in a spray bottle (CitoSpray), dry it and keep until the staining process. Cytological sample may be fixated and kept until staining by immersing into 95% alcohol solution (Histanol 95) for a minimum of 30 minutes.
2. Using liquid-based cytology method (LBC) and brush for collecting cytological samples, fixate the sample immediately (CitoFix, CitoFix in transport containers) by removing the brush head and immersing it in the fixative. At the beginning of processing the sample, isolate the cells from the fixative (one of the methods is to centrifuge the fixative) and place them on the microscope slide equally in a single layer. Cytological sample prepared in such a way is ready for staining.

#### The Papanicolaou staining method, **PROGRESSIVE**

The first stage of staining procedure depends on the method the cytological sample was collected and fixated on the microscope slide.

If the sample is dry and previously fixed using CitoSpray, it is necessary to keep it in a 95% alcohol solution (Histanol 95) for 10 minutes in order to remove polyglycols. If the section was fixated with a 95% alcohol solution (Histanol 95), ignore this step. During staining cytology samples (prepared by using the liquid based cytology method (LBC)) that contain low concentration of alcohol, rehydration by descending series of alcohol solutions is not necessary. The procedure starts by rinsing the section using distilled (demi) water and is then stained using Hematoxylin HP, Pap 1A/1B reagent.

#### Note: shake before use

1.	Rehydrate in descending series of alcohols (Histanol 95, Histanol 70) and in distilled (demi) water	10 dips in each of the 3 exchanges
2.	Stain using Hematoxylin HP, Pap 1A reagent	30 seconds or 2-3 min
	Note: Longer exposure of the section to Hematoxylin HP Pap 1A reagent may also stain cytoplasm (apart from nucleus). 30 seconds - for lighter cytoplasm staining 2-3 minutes - for darker cytoplasm staining	
3.	Rinse the section with tap or distilled water	30 seconds
4.	Blue using Scott's solution or Bluing reagent	1 min
5.	Note: If the mentioned reagents are not available, the section should be blued using indirect stream of water	3-5 minutes
6.	Dehydrate in ascending series of alcohols (Histanol 70 and Histanol 95)	10 dips in each of the 2 exchanges
7.	Stain using OG-6, Pap 2A reagent	2 min
8.	Rinse using 95% alcohol in two exchanges (Histanol 95)	30 seconds during each of the 2 exchanges
9.	Staining with EA 65 Pap 3D reagent	4 min
10.	Rinse using 95% alcohol in <u>two</u> exchanges (Histanol 95)	1 minutes in each of the 2 exchanges
11.	Dehydrate in 100% alcohol in <u>two</u> exchanges (Histanol 100)	1 minutes in each of the 2 exchanges
12.	Clear the section in xylene (BioClear) or in xylene substitute (BioClear New) in <u>two</u> exchanges	2 minutes in each of the 2 exchanges

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.

## Papanicolaou staining method, **REGRESSIVE**

The regressive staining method creates a better sample differentiation and clearer nuclear structure visibility.

The first stage of staining procedure depends on the method the cytological sample was collected and fixated on the microscope slide.

If the sample is dry and previously fixed using CitoSpray, it is necessary to keep it in a 95% alcohol solution (Histanol 95) for 10 minutes in order to remove polyglycols. If the section was fixated with a 95% alcohol solution (Histanol 95), ignore this step. During staining cytology samples (prepared by using the liquid based cytology method (LBC)) that contain low concentration of alcohol, rehydration by descending series of alcohol solutions is not necessary. The procedure starts by rinsing the section using distilled (demi) water and is then stained using Hematoxylin HP, Pap 1A or 1B.

### Note: shake before use

1.	Rehydrate in descending series of alcohols (Histanol 95, Histanol 70) and in distilled (demi) water	10 dips in each of the 3 exchanges
2.	Stain using Hematoxylin HP, Pap 1A reagent	4 min
3.	Rinse the section with tap or distilled water	30 seconds
4.	Differentiate using HCL Pap reagent or in 0.1% HCl solution	5-10 seconds
	Note: This step removes excessive hematoxylin from the nucleus and cytoplasm. Discoloration of the nuclei can occur if the section is treated with the differentiation agent for too long.	
5.	Rinse the section with tap or distilled water	10 dips
6.	Blue using Scott's solution or Bluing reagent	1 min
	Note: If the mentioned reagents are not available, the section should be blued using indirect stream of water	3-5 minutes
7.	Dehydrate in ascending series of alcohols (Histanol 70 and Histanol 95)	10 dips in each of the 2 exchanges
8.	Stain using OG-6, Pap 2A reagent	2 min
9.	Rinse using 95% alcohol in two exchanges (Histanol 95)	30 seconds during each of the 2 exchanges
10.	Staining with EA 65 Pap 3D reagent	4 min
11.	Rinse using 95% alcohol in <u>two</u> exchanges (Histanol 95)	1 minutes in each of the 2 exchanges
12.	Dehydrate in 100% alcohol in <u>two</u> exchanges (Histanol 100)	1 minutes in each of the 2 exchanges
13.	Clear the section in xylene (BioClear) or in xylene substitute (BioClear New) in <u>two</u> exchanges	2 minutes in each of the 2 exchanges

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.

### Note

In the case of subsidence in the Hematoxylin HP, Pap 1A/1B solution or formation of metallic glow on the surface, reagent should be filtered before use. Time periods of staining procedures are not completely standardized. The suggested methods are in accordance with BioGnost's reagents' properties and correspond to longtime clinical and laboratory practice. Intensity of staining depends on the period of exposure to stains and reagents. Staining procedure can be changed according to personal preferences if they correspond to the basic principles of cytotechnology.

### Result

Blue-green to blue-grey - cyanophilic (basophilic) cytoplasm

Pink to red-purple - eosinophilic (acidophilic) cytoplasm

Pink-red - keratinized cytoplasm

Reddish - erythrocytes

Blue to dark purple - nuclei

Grey-blue - microorganisms

Grey-green - *Trichomonas*

### 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.

### 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 EA 65, Pap 3D reagent in a tightly closed original package at temperature between 15°C and 25°C. Keep in dry 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. Papanicolaou, G.N. (1941): Some improved methods for staining vaginal smears. J Lab Clin Med.
2. Papanicolaou, G.N. (1942): A new procedure for staining vaginal smears. Science.
3. Carson, F.L., Hladik C. (2009): Histotechnology: A self-instructional text, 3<sup>rd</sup> ed. ASCP Press.

EA65-OT-X, V7-EN6, 2 April 2019, AK/VR

	Refer to the supplied documentation		Storage temperature range		Number of tests in package		Product code		European Conformity
	Refer to supplied instructions		Keep away from heat and sunlight		Valid until		Lot number		Manufacturer
	For in vitro diagnostic use only		Keep in dry place		Caution - fragile				

 BIOGNOST Ltd.  
Medjugorska 59  
10040 Zagreb  
CROATIA  
www.biognost.com

