BIOGNOST®

EA 65 REAGENT, PAP 3C

IVD *In vitro* diagnostic medical device CE

Cytoplasmic staining reagent acc. to Papanicolaou - red hue

Polychromatic counterstain for gynecological and non-gynecological samples in cytology

INSTRUCTIONS FOR USE

REF Catalogue number: EA65C-OT-100 (100 mL) EA65C-OT-500 (500 mL) EA65C-OT-1L (1000 mL) EA65C-OT-2.5L (2500 mL)

Introduction

EA 65 reagent, Pap 3C reagent is an alcoholic solution of two acid dyes, Eosin Y and Light Green SF, with added Bismarck Brown dye and 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 reagent and one of the polychromatic EA reagent formulations. The Orange G molecule stains the cytoplasm, and in later stages of the procedure it remains only in the mature, keratinized cells. The third step consists of using of one of the polychromatic EA solutions that stains the unstained cellular components, such as squamous cells, nucleoli, cilia, and erythrocytes. Test samples can be gynecological and non-gynecological, such as sputum, urine, and cytological puncture samples. In order to obtain optimal staining results, EA 65 Pap 3C reagent has properties completely in compliance with other BioGnost's reagents for cytological smearing acc. to Papanicolaou - Hematoxylin HP, Pap 1A reagent and OG-6, Pap 2A reagent.

Product description

EA 65 REAGENT, PAP 3C - Polychromatic counterstain for staining gynecological and 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 cytoplasms of hormonally different cells. Concentration and interrelation between Eosin Y and Light Green SF dyes are what EA 31 differs from other BioGnost's EA Pap reagents. Contains small amount of green dye from EA31 formulation and is used for lighter and more transparent cytoplasmic staining.

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 begins by rinsing the section using distilled (demi) water and is then stained using Hematoxylin HP, Pap 1A.

Note: shake well all reagents before use

1.	Rehydrate in descending series of alcohols (Histanol 95 and Histanol 70) and in distilled or demineralized water	10 dips in each of the 3 exchanges
2.	Stain using Hematoxylin HP, Pap 1A reagent	30 seconds or 2-3 minutes
	Note: with longer incubation time, there is a greater possibility of staining cytoplasm together with the nuclei 30 seconds – light cytoplasm staining 2-3 minutes – strong cytoplasm staining	
3.	Rinse in distilled/demineralized water	30 seconds
4.	Blue using Scott's solution or Bluing reagent	1 minute
	Note: If the mentioned reagents are not available, the section should be blued using indirect stream of water	3-5 minutes
5.	Dehydrate in ascending series of alcohols (Histanol 70, and Histanol 95)	10 dips in each of the 2 exchanges
6.	Stain using OG-6 reagent, Pap 2A	2 minutes
7.	Rinse using 95% alcohol in two exchanges (Histanol 95)	30 seconds in each of the 2 exchanges
8.	Stain using EA 65 reagent, Pap 3C	4 minutes
9.	Rinse and dehydrate using 95% alcohol in two exchanges (Histanol 95)	1 minute in each of the 2 exchanges
10.	Dehydrate using 100% alcohol in two exchanges (Histanol 100)	1 minute in each of the 2 exchanges
11.	Clear the section in two exchanges of xylene (BioClear) or xylene substitute (BioClear New)	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 reagent.

Note: shake well all reagents before use

1.	Rehydrate in descending series of alcohols (Histanol 95, and Histanol 70) and in distilled or demineralized water	10 dips in each of the 3 exchanges
2.	Stain using Hematoxylin HP, Pap 1A reagent	4 minutes
3.	Rinse in distilled/demineralized water	30 seconds
4.	Differentiation 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 exposed to differentiation agent for too long.	
5.	Rinse in distilled or tap water	10 dips
6.	Blue using Scott's solution or Bluing reagent	1 minute
	Note: If the mentioned reagents are not available, the section should be blued using indirect stream of water	3-5 min
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 reagent, Pap 2A or Orange II reagent, Pap 2B	2 minutes
9.	Rinse using 95% alcohol in two exchanges (Histanol 95)	30 seconds in each of the 2 exchanges
10.	Stain using EA 65 reagent, Pap 3C	4 minutes
11.	Rinse and dehydrate using 95% alcohol in two exchanges (Histanol 95)	1 minute in each of the 2 exchanges
12.	Dehydrate using 100% alcohol in two exchanges (Histanol 100)	1 minute in each of the 2 exchanges
13.	Clear the section in two exchanges of xylene (BioClear) or xylene substitute (BioClear New)	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 reagent or formation of metallic glow on the surface, reagent should be filtrated 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.

Results

Pink-red - keratinized cytoplasm, cyanophilic (basophilic) cytoplasm and eosinophilic (acidophilic) cytoplasm
Reddish-brown - 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 3C 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, 3rd ed. ASCP Press.

EA65C-X, V10-EN8, January 23, 2024, IŠP/VR

⚠	Refer to the supplied documentation	°C	Storage temperature range	Σ	Number of tests in package	REF	Product code	CE	European Conformity	BIOGNOST Ltd. Medjugorska 59 10040 Zagreb CROATIA www.biognost.com	CE
i	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						