

EA 65 REAGENT, PAP 3C

IVD *In vitro* diagnostic medical device



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 cytoplasm 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.

1.	Rehydrate in descending series of alcohols (Histanol 95, Histanol 80 and Histanol 70) and in distilled or demineralized water	6-8 dips in each of the 4 exchanges
2.	Stain using Hematoxylin HP	15 seconds to 3 min
3.	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
4.	Immerse the sections in distilled/demineralized water.	
5.	Dehydrate in ascending series of alcohols (Histanol 70, Histanol 80 and Histanol 95)	6-8 dips in each of the 3 exchanges
6.	Stain using OG-6 reagent or Orange II Pap reagent	2-3 minutes
7.	Rinse using 95% alcohol in two exchanges (Histanol 95)	6-8 dips in each of the 2 exchanges
8.	Stain using EA 65 reagent, Pap 3C or EA 65 reagent, Pap 3D.	2-3 minutes
9.	Rinse using 95% alcohol (Histanol 95)	6-8 dips
10.	Dehydrate using 100% alcohol (Histanol 100)	6-8 dips
11.	Dehydrate using 100% alcohol (Histanol 100)	3-5 minutes
12.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	6-8 dips
13.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3-5 minutes

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.

1.	Rehydrate in descending series of alcohols (Histanol 95, Histanol 80 and Histanol 70) and in distilled or demineralized water	6-8 dips in each of the 4 exchanges
2.	Stain using Hematoxylin HP, Pap 1A reagent	6 min
3.	Rinse in distilled/demineralized water	6-8 dips
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 treated with the differentiation agent for too long.	
5.	Rinse in distilled water	6-8 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	
7.	Immerse the sections in distilled/demineralized water.	
8.	Dehydrate in ascending series of alcohols (Histanol 70, Histanol 80 and Histanol 95)	6-8 dips in each of the 3 exchanges
9.	Stain using OG-6 reagent, Pap 2A or Orange II reagent, Pap 2B	3 min
10.	Rinse using 95% alcohol in two exchanges (Histanol 95)	6-8 dips in each of the 2 exchanges
11.	Stain using EA 65 reagent, Pap 3C or EA 65 reagent, Pap 3D.	3 min
12.	Rinse using 95% alcohol (Histanol 95)	6-8 dips
13.	Dehydrate using 100% alcohol (Histanol 100)	6-8 dips
14.	Dehydrate using 100% alcohol (Histanol 100)	3-5 min
15.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	6-8 dips
16.	Clear the section in xylene (BioClear) or in a xylene substitute (BioClear New)	3-5 min

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 solution 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*

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, V6-EN6, 2 April 2019, IŠP/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 <i>in vitro</i> diagnostic use only		Keep in dry place		Caution - fragile				

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