

# ORANGE II REAGENT, PAP 2B

IVD *In vitro* diagnostic medical device



## Cytoplasmic staining reagent acc. to Papanicolaou Counterstain for monochromatic staining of samples in cytology

### INSTRUCTIONS FOR USE

REF Catalogue number: OR2-OT-100 (100 mL) OR2-OT-500 (500 mL) OR2-OT-1L (1000 mL) OR2-OT-2.5L (2500 mL)

#### Introduction

Orange II reagent, Pap 2B a solution of Orange II dye 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 contrast staining using the monochromatic OG-6 or Orange II reagents and one of the polychromatic EA reagent formulations consisting of two acid dyes, the Eosin Y and Light Green SF. The Orange II molecule stains the cytoplasm, and in later stages of the procedure it remains only in the mature, keratinized cells that turn different shades of orange. The third step consists of using 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, BioGnost's Orange II reagent, Pap 2B properties are completely in accordance with other BioGnost's reagents used for cytological staining acc. to Papanicolaou - Hematoxylin HP, Pap 1A and 1B, EA 31 reagent, Pap 3A, and EA50 reagent, Pap 3B, as well as alternative counterstain polychromatic stains, such as EA 65 reagent, Pap 3C and 3D.

#### Product description

**ORANGE II REAGENT, PAP 2B** - Counterstain for monochromatic staining of samples in exfoliative cytology. Contains BSC-certified Orange II dye with added phosphotungstic acid and necessary stabilizers.

#### 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.

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 or 1B reagent	2-3 minutes
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 section 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, Pap 2A or Orange II reagent, Pap 2B	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 31, Pap 3A reagent or EA 50, Pap 3B reagent	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/1B 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 or 1B 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	3-5 minutes
7.	Immerse the section 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 31, Pap 3A reagent or EA 50, Pap 3B reagent	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 minutes
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 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.

### 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.

### Results

Grey-blue - microorganisms

Grey-green - *Trichomonas*

Blue to dark purple - nuclei

Yellow-orange - keratinized cells

Red - erythrocytes

Pink - eosinophilic (acidophilic) cells

Staining with EA31 reagent, Pap 3A: Blue-green to green - cytoplasm of cyanophilic (basophilic) cells

Staining with EA50 reagent, Pap 3B: Blue-green - cytoplasm of cyanophilic (basophilic) cells

### 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 Orange II reagent, Pap 2B 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.
4. Sherwani, R.K., Khaqn, T. et al. (2007): Conventional Pap Smear and Liquid Based Cytology for Cervical Cancer Screening – A Comparative Study, Journal of Cytology, 24 (4): pp 167-172.

OR2-OT-X, V3-EN3, 31 May 2017, 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 <i>in vitro</i> diagnostic use only		Keep in dry place		Caution - fragile				

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

