BIOGNOST®

EA 50 REAGENT, PAP 3B

CE IVD *In vitro* diagnostic medical device Classified acc. to Regulation (EU) 2017/746 - **Class A** device

Polychromatic reagent for cytoplasmic staining acc. to Papanicolaou

INSTRUCTIONS FOR USE

BASIC UDI number	385889212HPC30708STARVF				
EMDN code	W01030708				
REF **Cat. number**	**Volume**	**UDI-DI number**	REF **Cat. number**	**Volume**	**UDI-DI number**
EA50-OT-100	100 mL	03858888828745	EA50-OT-2.5L	2500 mL	03858888821968
EA50-OT-500	500 mL	03858888821920	EA50-OT-5L	5000 mL	03858892120484
EA50-OT-1L	1000 mL	03858888821937	EA50-OT-20L	20000 mL	03858890001853

Intended use and test principle

EA 50 reagent, Papa 3B is an alcoholic solution of two acid dyes, pink Eosin Y and green, with the addition of phosphotungstic acid (PTA). The first step of the Papanicolau staining method is to stain the nuclei with a haematoxylin solution, and the following two steps: counterstaining with monochromatic OG-6 and one of the formulations of polychromatic EA reagents. Orange G molecule stains the cytoplasm and subsequently remains only in mature, keratinized cells. The third step uses one of the polychromatic EA solutions which stains the unstained elements of the cell, such as: squamous cells, nucleoli, flagella and erythrocytes. Test samples can be gynaecological and non gynecological, such as saliva, urine or aspirations. In order to achieve optimal staining results, EA 50 reagent, Papa 3B's characteristics are completely adapted to other BioGnost reagents for staining according to Papanicolau staining method – Haematoxylin HP, Pap 1A and OG-6 reagent, Pap 2A.

Product description

- **EA 50 REAGENT, PAP 3B** – polychromatic reagent for staining of gynaecological samples in cytology. It contains pink Eosin Y and green dye with phosphotungstic acid and necessary stabilizers. It differs from other BioGnost EA Pap reagents by concentration and Eosin Y and green dye ratio.

An example of EA 50 REAGENT, PAP 3B application

Additional reagents and materials that can be used in the staining method

- Fixative agents such as BioGnost's Citospray solution
- Dehydration/rehydration agents such as BioGnost's alcohol solutions: Histanol 70, Histanol 80, Histanol 95, and Histanol 100
- Clearing agents, such as BioClear xylene or BioClear New, an aliphatic hydrocarbon-based xylene substitute
- Microscopic slide covering agents and cover glass mountants such as BioGnost's BioMount, BioMount High, BioMount M, BioMount New, BioMount New Low, BioMount DPX, BioMount DPX High, BioMount DPX Low, BioMount DPX New, BioMount C, BioMount Aqua
- VitroGnost slides and coverslips for use in histopathology and cytology
- BioGnost's immersion oils, types A, C, FF, 37 or Tropical Grade
- BioGnost Papanicolaou staining reagents: Hematoxylin HP, Pap 1A and OG-6, Pap 2A reagent

Preparation of cytological smear for staining

There are two ways to collect and prepare cytological samples:

1. Use a swab to collect the cytological sample and apply to a glass slide (VitroGnost), immediately fix using a fixative in a spray bottle (CitoSpray), dry and keep until the staining procedure begins. A cytological sample can also be fixed and kept until staining by immersing in 95% alcohol solution (Histanol 95) for at least 30 minutes.
2. Using LBC (Liquid-Based Citology) use a brush for cytological sample collection. Fix the sample immediately (CitoFix, CitoFix in transport containers) by removing the head of the brush and immersing it in a fixative. At the start of the smear processing, separate the cells from the fixative liquid (one of the ways to do this is to centrifuge the fixative) and apply them to a slide so that the cells are spread evenly in one layer. A cytological smear prepared like this is ready for staining.

Cytological smear staining according to Papanicolau staining method

The beginning of the staining method depends on the manner of collection and fixation of the sample.

If the sample is dry and previously fixed using CitoSpray, before staining it is necessary to leave the sample in 95% alcohol (Histanol 95) to remove polyglycols. If the sample is fixed using 95% alcohol, this step is not necessary. When staining cytological samples prepared using the Liquid-Based Cytology method (LBC), which contain a low concentration of alcohol, rehydration using a descending series of alcohol is not necessary. The procedure begins with rinsing the sample with distilled/demineralized water and continues by staining with Haematoxylin HP, Papa 1A.

NOTE

Apply the reagent so it completely covers the slide.

A) progressive staining method

1.	Rehydrate in descending series of alcohol (Histanol 95 and Histanol 70) and in distilled/demineralized water	3 exchanges, 10 dips each
2.	Stain using Haematoxylin HP, Pap 1A reagent	30 seconds
	Note: extended exposure of sample to Haematoxylin HP, Pap 1A reagent can also stain the cytoplasm blue	
3.	Rinse in distilled/demineralized water	30 seconds
4.	Stain blue using Scott's solution or the Bluing reagent	1 min
	Note: if the aforementioned reagents are unavailable, the sample can be blued under an indirect stream of tap water	3-5 min
5.	Dehydrate in ascending series of alcohol (Histanol 70 and Histanol 95)	2 exchanges, 10 dips each
6.	Stain using OG-6, Pap 2A reagent	2 min
7.	Rinse with 95% alcohol, two exchanges (Histanol 95)	2 exchanges, 30 seconds each
8.	Stain using EA 50, Pap 3B reagent	4 min
9.	Rinse and dehydrate in 95% alcohol, two exchanges (Histanol 95)	2 exchanges, 1 min each
10.	Dehydrate in 100% alcohol, two exchanges (Histanol 100)	2 exchanges, 1 min each
11.	Clear in xylene (BioClear) or xylene substitute (BioClear New), two exchanges	2 exchanges, 2 min each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium. If BioClear xylene was used, use one of BioGnost's xylene-based mountants (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate mountant is BioMount New. Cover the slide with a VitroGnost cover glass.

B) regressive staining method

The regressive staining method provides a better differentiation of the sample and better visibility of nuclear structures.

1.	Rehydrate in descending series of alcohol (Histanol 95 and Histanol 70) and in distilled/demineralized water	3 exchanges, 10 dips each
2.	Stain using Haematoxylin HP, Pap 1A reagent	4 min
3.	Rinse in tap water or distilled/demineralized water	30 seconds
4.	Differentiate using HCL Pap reagent or 0.1% HCl solution.	5-10 seconds
	Note: this step removes an excess of haematoxylin from nucleus and cytoplasm. If the sample is treated with the differentiating agent, the dye on the nuclei can wash away	
5.	Rinse in tap water or distilled/demineralized water	10 dips
6.	Stain blue using Scott's solution or the Bluing reagent	1 min
	Note: if the aforementioned reagents are unavailable, the sample can be blued under an indirect stream of tap water	3-5 min
7.	Dehydrate in ascending series of alcohol (Histanol 70 and Histanol 95)	2 exchanges, 10 dips each
8.	Stain using OG-6, Pap 2A reagent	2 min
9.	Rinse and dehydrate in 95% alcohol, two exchanges (Histanol 95)	2 exchanges, 30 sec each
10.	Stain using EA 50, Pap 3B reagent	4 min
11.	Rinse and dehydrate in 95% alcohol, two exchanges (Histanol 95)	2 exchanges, 1 min each
12.	Dehydrate in 100% alcohol, two exchanges (Histanol 100)	2 exchanges, 1 min each
13.	Clear in xylene (BioClear) or xylene substitute (BioClear New), two exchanges	2 exchanges, 2 min each

Immediately after clearing, apply an appropriate BioMount covering/mounting medium. If BioClear xylene was used, use one of BioGnost's xylene-based mountants (BioMount, BioMount High, BioMount M, BioMount DPX, BioMount C, or universal BioMount New). If BioClear New xylene substitute was used, the appropriate mountant is BioMount New. Cover the slide with a VitroGnost cover glass.

Note

If Haematoxylin HP, Pap 1A reagent shows sedimentation or metallic sheen, filter before use.

Result

Nuclei – blue
Keratinized cells – orange-yellow
Superficial epithelial squamous cells, erythrocytes, nucleoli, flagella - red – pink
Cytoplasm of all other types of cells ((parabasal and intermediate squamous cells, cylindrical cells, polymorphonuclear leukocytes, lymphocytes, histiocytes, adenocarcinoma and undifferentiated carcinoma cells) – green

Limitations

This product is intended for professional laboratory use for diagnostic purposes only. Deviations from the described staining procedure may cause variations in results.

Sample preparation and diagnostics

Use only appropriate instruments for collecting and preparing the samples. Process the samples using modern technology and mark them clearly. It is necessary to follow the manufacturer's instructions for use. To avoid errors, staining of the samples and diagnosis may only be performed by qualified personnel. Use a microscope that complies with medical diagnostic laboratory standards.
If a serious incident occurs during use of this product or as a result of its use, please report it to the manufacturer or authorized representative and competent authority.

Safety at work and environmental protection

Handle the product in accordance with occupational health and environmental protection guidelines. Used and expired solutions must be disposed of as special waste following national guidelines. Reagents used in this procedure can pose a danger to human health. The examined tissue samples are potentially infectious, therefore it is necessary to implement human health protection measures in accordance with good laboratory practice guidelines. It is mandatory to read and act according to the information and warning signs printed on the product label, instructions for use and in the safety data sheet, which is available on request.

Storage, stability, and shelf life

Upon receipt, store the product in a dry place and well-closed original packaging at a temperature of +15 °C to +25 °C. Do not freeze or expose to direct sunlight. After first opening, the product can be used until the specified expiry date, if stored properly. The production date and expiration date are printed on the product label.

Reference

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.

Warnings and precautions regarding the materials contained in the product:

H225	Highly flammable liquid and vapor
H302	Harmful if swallowed.
P210	Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P233	Keep container tightly closed.
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P301+P312	IF SWALLOWED: call a POISON CENTER/doctor if you feel unwell
P501	Dispose of contents/container in accordance with local regulations.

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	Manufacturer	LOT	Batch code		Consult instructions for use	CE	European conformity
	Date of manufacture	REF	Catalogue number		Caution	UDI	Unique device identifier
	Use-by date	°C	Temperature limit	IVD	*In vitro* diagnostic medical device		

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Version	Description / reason for change	Date
16	Revised in acc to Regulation (EU) 2017/746 - IVDR	13.01.2026.