BIOGNOST®

EA 31 REAGENT, PAP 3A

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-DI	385889212HPC30708STARVF				
EMDN code	W01030708				
REF **Catalog number**	**Volume**	**UDI-DI**	REF **Catalog number**	**Volume**	**UDI-DI**
EA31-OT-100	100 mL	03858890000351	EA31-OT-1L	1000 mL	03858888821890
EA31-OT-500	500 mL	03858888821883	EA31-OT-2.5L	2500 mL	03858888821876

Intended use and test principle

EA 31 reagent, Pap 3A is an alcoholic solution of two acidic dyes, pink Eosin Y and green, with the addition of phosphotungstic acid (PTA). The first step of Papanicolaou staining involves staining the nuclei with a hematoxylin solution, and the next two steps are contrast staining with monochromatic OG-6 and one of the formulations of polychromatic EA reagents. The Orange G molecule stains the cytoplasm, and during further processing it remains only in mature, keratinized cells. In the third step, one of the polychromatic EA solutions is used to stain unstained parts of the cell such as squamous cells, nucleoli, flagella, and erythrocytes. Samples for testing can be gynecological and non-gynecological, such as sputum, urine, samples obtained by cytological puncture. In order to obtain optimal staining results, EA 31 reagent, Pap 3A is fully compatible with other BioGnost reagents for cytological staining according to the Papanicolau method – Hematoxylin HP, Pap 1A reagent and OG-6 reagent, Pap 2A.

Product description

- **EA 31 REAGENT, PAP 3A** - Contrast stain for polychromatic staining of gynecological samples in cytology. Contains BSC-certified dyes Eosin Y and Light Green SF with phosphotungstic acid and necessary stabilizers. It differs from other BioGnost EA Pap reagents in concentration and ratio of Eosin Y and Light Green SF dye.

Example of EA 31 reagent, PAP 3A use

Additional reagents and materials that can be used in the method

- A fixative 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
- Immersion oils such as BioGnost's Immersion Oil, Immersion Oils types A, C, FF, 37, or Immersion Oil Tropical Grade
- BioGnost reagents for Pap staining: Hematoxylin HP, Pap 1A and OG-6, Pap 2A reagent

Preparation of cytological smears for staining

There are two methods for collecting and preparing cytological samples:

1. After collecting the cytological sample by swab, apply it to a slide (VitroGnost), immediately fix it with the fixative from the spray bottle (CitoSpray), dry, and store until the staining procedure. The cytological sample can also be fixed and stored until staining immersed in a 95% alcohol solution (Histanol 95) for a minimum of 30 minutes.
2. Using the method of liquid cytology (LBC, Liquid-Based Cytology) using a brush for taking cytological samples, immediately fix the sample by separating the brush head and immersing it in a fixative. At the beginning of the cytological sample processing, separate the cells from the fixation fluid (one way is to centrifuge the fixation fluid) and apply them to the glass in such a way that the cells are evenly distributed in one layer. The cytological sample prepared this way is ready for staining.

Procedure for staining cytological samples according to the Papanicolaou method

The start of the staining process depends on the way in which the cytological sample was collected and fixed to the microscopy slide.

If the sample is dry and previously fixed with CitoSpray, it should be kept in 95% alcohol (Histanol 95) for 10 minutes before staining to remove polyglycol. If the preparation is fixed with a 95% alcohol solution (Histanol 95), skip this step. During the procedure of staining cytological samples prepared by the liquid cytology method (LBC) containing a low concentration of alcohol, rehydration with a descending series of alcohol solutions is not necessary. The procedure begins with rinsing the preparation with distilled (demi) water, and continues with the process of staining with Hematoxylin HP, Pap 1A.

Note

Apply the reagent so that it completely covers the section.

A) progressive staining method

1.	Rehydrate in descending order of alcohol (Histanol 95 and Histanol 70) and in distilled/demineralized water	3 exchanges of 10 dips
2.	Stain with Hematoxylin HP, Pap 1A reagent	30 sec
	Note: Prolonged exposure of the preparation to Hematoxylin HP, Pap 1A reagent may stain the cytoplasm in addition to the nucleus.	
3.	Rinse in distilled/demineralized or tap water	30 sec
4.	Make nuclei turn blue using Scott's solution or Bluing reagent	1 min
	Note: In the absence of the aforementioned reagents, rinse the preparation under an indirect stream of running water	3-5 min
5.	Dehydrate in ascending order of alcohol (Histanol 70 and Histanol 95)	2 exchanges of 10 dips
6.	Stain with OG-6, Pap 2A reagent	2 min
7.	Rinse in 95% alcohol through two exchanges (Histanol 95)	2 exchanges, 30 sec each
8.	Stain with EA 31, Pap 3A reagent	4 min
9.	Rinse and dehydrate in 95% alcohol through two exchanges (Histanol 95)	2 exchanges of 1 min each
10.	Dehydrate in 100% alcohol through two changes (Histanol 100)	2 exchanges of 1 min each
11.	Clear in xylene (BioClear) or xylene substitute through two exchanges (BioClear New)	2 exchanges of 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 section with a VitroGnost cover glass.

B) regressive staining method

With the regressive staining method, better differentiation of the sample and clearer visibility of nuclear structures is achieved

1.	Rehydrate in descending order of alcohol (Histanol 95 and Histanol 70) and in distilled/demineralized water	3 exchanges of 10 dips

2.	Stain with Hematoxylin HP, Pap 1A reagent	4 min
3.	Rinse in distilled/demineralized or tap water	30 sec
4.	Differentiation with HCl Pap reagent or in 0.1% HCl solution	5-10 sec
	Note: This step removes excess hematoxylin from the nucleus and cytoplasm. If the sample has been treated with a differentiating agent for too long, the nuclei may become discolored	
5.	Rinse in distilled/demineralized or tap water	10 dips
6.	Bluing of nuclei with Scott's solution or Bluing reagent	1 min
	Note: In the absence of the aforementioned reagents, rinse the preparation under an indirect stream of running water.	3-5 min
7.	Dehydrate in ascending order of alcohol (Histanol 70 and Histanol 95)	2 exchanges of 10 dips
8.	Stain with OG-6, Pap 2A reagent	2 min
9.	Rinse in 95% alcohol through two exchanges (Histanol 95)	2 exchanges, 30 sec each
10.	Stain with EA 31, Pap 3A reagent	4 min
11.	Rinse and dehydrate in 95% alcohol through two exchanges (Histanol 95)	2 exchanges of 1 min each
12.	Dehydrate in 100% alcohol through two changes (Histanol 100)	2 exchanges of 1 min each
13.	Clear in xylene (BioClear) or xylene substitute through two exchanges (BioClear New)	2 exchanges of 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 section with a VitroGnost cover glass.

Note

If precipitation occurs in the Hematoxylin HP, Pap 1A solution or if a metallic sheen has formed on the surface, the reagent must be filtered before use.

Result

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

Limitations

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

References

1. Carson, FL, Hladik C. (2009): Histotechnology: A self-instructional text, 3rd ed. ASCP Press.
2. Papanicolaou, G.N. (1941): Some improved methods for staining vaginal smears. J Lab Clin Med.
3. Papanicolaou, G.N. (1942): A new procedure for staining vaginal smears. Science.
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): p. 167-172.

Warnings and precautions regarding the materials contained in the product:

H225	Highly flammable liquid and vapour.
H302	Harmful if swallowed.
P210	Keep away from heat, sparks, open flames and other ignition sources. Do not smoke.
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/regional/national/international 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
12	Revised acc. to Regulation (EU) 2017/746 - IVDR	09/04/2026