KIT FOR RAPID PAP STAINING

IVD In vitro diagnostic medical device CE

Ready-To-Use kit for rapid cytology smears staining acc. to Papanicolaou, contains medium for permanent section covering

INSTRUCTIONS FOR USE

REF Catalogue number: PAP-100T (for 100 tests)

Introduction

Ready-To-Use kit for cytology gynecology and non-gynecology smears staining according to Papanicolaou enables complete sample processing in just a few minutes after sampling. The staining procedure is simplified and conducted in a few minutes. It contains all the reagents necessary for sample processing - 95% alcohol as fixative, deionized water, Hematoxylin HP, nuclear bluing reagent, OG-6 reagent and EA 50 reagent, as well as 100% alcohol for tissue dehydration and BioClear New (xylene substitute) for section clearing. It also contains mounting medium of very low viscosity and optimal refractive index (BioMount New) in practical packaging. The reagents are placed in practical jars and sections may be directly immersed. They are placed in the order of use in the box, which lowers the possibility of contamination of reagents during staining. The kit contains additional jar of Bluing reagent used after staining 50 sections. The kit is sufficient for staining approximately 100 smears.

Product description

- **KIT FOR RAPID PAP STAINING** – Eight-reagent kit (in 20 jars) for rapid progressive gynecology and non-gynecology cytology samples staining. Contains additional medium for permanent section covering.

The kit contains:	Amount and volume
Histanol 95	7 x 70 mL
Deionized water	3 x 70 mL
Hematoxylin HP, Pap 1A	1 x 70 mL
Bluing reagent	2 x 70 mL
OG-6 reagent, Pap 2A	1 x 70 mL
EA 50 reagent, Pap 3B	1 x 70 mL
Histanol 100	2 x 70 mL
BioClear New	3 x 70 mL
BioMount New Low	2 x 10 mL

Other slides and reagents that may be used in staining

- High-quality glass slides for use in histopathology and cytology, such as VitroGnost SUPER GRADE or one of more than 30 models of BioGnost's glass slides
- VitroGnost cover glass, dimensions range from 18x18 mm to 24x60 mm

Note: The jars are placed and marked in order of use. Open the jars before staining. Immediately after finishing the staining process, close the jars using screw caps and close tightly in order to prevent evaporation.

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.

Sample staining procedure

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.

Start the staining process beginning from jar 1 containing Histanol 95. Continue with staining until the end (jar 19)

Change deionized water regularly; change alcohols and BioClear New (xylene substitute) if necessary

Step	Jar		
1.	**1**	Treat with 95% alcohol (Histanol 95)	1 min
2.	**2**	Treat with 95% alcohol (Histanol 95)	10 dips
3.	**3**	Rinse in deionized water	10 dips
4.	**4**	Stain using Hematoxylin HP, Pap 1A reagent	30 sec
5.	**5**	Rinse in deionized water	10 dips
6.	**6**	Nuclear bluing with Bluing reagent	1 min
		Note: replace Bluing reagent with a fresh one (part of the kit) after each 50 sections	
7.	**7**	Rinse in deionized water	10 dips
8.	**8**	Treat with 95% alcohol (Histanol 95)	10 dips
9.	**9**	Stain using OG-6, Pap 2B reagent	2 minutes

10.	**10**	Rinse using 95% alcohol (Histanol 95)	10 dips
11.	**11**	Rinse using 95% alcohol (Histanol 95)	10 dips
12.	**12**	Stain with EA50 reagent, Pap 3B	4 minutes
13.	**13**	Rinse using 95% alcohol (Histanol 95)	10 dips
14.	**14**	Rinse using 95% alcohol (Histanol 95)	10 dips
15.	**15**	Dehydrate using 100% alcohol (Histanol 100)	10 dips
16.	**16**	Dehydrate using 100% alcohol (Histanol 100)	10 dips
17.	**17**	Clear the section in BioClear New (xylene substitute)	10 dips
18.	**18**	Clear the section in BioClear New (xylene substitute)	10 dips
19.	**19**	Clear the section in BioClear New (xylene substitute)	1 min

Immediately after clearing apply an appropriate BioMount New medium for covering/mounting cover glass
Cover the section with a VitroGnost cover glass.

Results

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

Note

Time periods of staining processes are not entirely standardized and they approximately correspond to clinical and laboratory practical experience. Intensity of staining depends on the period of immersion in the dye. Real staining protocol depends on personal requests and priorities.

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 the kit in a tightly sealed original packaging 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. Carson, F.L., 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): pp 167-172.

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