IVD In Vitro Diagnostic Medical Device
For professional use only

Papanicolaou stains

35040 Papanicolaou's stain 0.G.6
35169 Papanicolaou's stain EA50

Cat. No	Pack Type	Pack Size
350405X	Plastic Bottle	1 l
351695T	Glass Bottle	1 l

Composition
Cat. No. 35040

C.I. 16230	1.9 g/l
$H_3[P(W_3O_{10})_4]$	0.1 g/l

Cat. No. 35169

Intended Use(s)
Staining solutions and dyes to differentiate in medical diagnosis suspected cells types in samples for cytological cancer, e.g. cervical cancer.
It is used for the initial evaluation to differentiate nuclei,cytoplama and squamous cells and examined under microsope
Evaluate the result by comparing it to what would be the age related normal values
Review of the samples helps in determining the need for ancillary studies.
An initial review ot the patient´s clinical background is necessary to use in conjunction with the result of the staining

Samples derived from the human body

References:
* Staining procedures (1981). CLARK, G.:
* Conn´s Biological stains 10th edition, R.W. Horobin, J.A. Kiernan

Principle
The most widely used staining procedure for cytological specimens is Papanicolaou's technique. In the first staining step the nuclei are stained by a haematoxylin solution. Nuclei are stained blue, dark violet to black.
The second staining step is cytoplasmic staining by orange staining solution, especially for demonstration of mature and keratinised cells. The target structures are stained orange in different intensities. In the third staining step the so-called polychromatic solution is used, a mixture of eosin, light green SF and Bismarck brown. The polychromatic solution is used for demonstration of differentiation of squamous cells
e.g. cervical cancer and cycle diagnosis for examination under microsope
Samples derived from the human body

Application
Papanicolaou's stain OG6 gives a pale, yellow-orange staining result with mature and keratinised squamous cells. Papanicolaou's solution 2b, Orange II solution gives a more intense reddish staining result with mature and keratinised squamous cells.

Sample material and preparation
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Gynaecological and non-gynaecological specimen as sputum, urine, FNAB, body effusions, lavages
Samples derived from the human body.
The collected cells are smeared on a microscope slide and immediately wet fixed with a thin film to maximize cell preservation
In order to avoid errors, the staining process must be carried out by an expert.
National guidelines for work safety and quality assurance must be followed.
Microscopes equipped according to the standard must be used.
If necessary use a centrifuge suitable for medical diagnostic laboratory.

Fixation
Wet fixation immediately with Cytology spray fixative or wet fixation immediately in 96% ethanol for minimum 30 min.
All samples must be clearly labelled.
Suitable instruments must be used for taking samples and their preparation; manufacturer instructions for application /use must be followed.

Reagent

Cat. No	Description	Pack Size
35040	Papanicolaou's stain OG6	1 l
35169	Papanicolaou's solution EA50	1 l
35194	Haematoxylin Harris' mercury free	1 l

Procedure
Wet fixed samples on microscope slides
Conventional staining, manual:

1. Wash with 96 % alcohol*.
2. Wash with 80 % alcohol*.
3. Wash with 70 % alcohol*.
4. Wash with 50 % alcohol*.

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5. Wash with distilled water
6. Stain in Harris' haematoxylin solution 3 min
7. Rinse under weak stream of tap water 3-5 min
8. Wash with 50 % alcohol
9. Wash with 70 % alcohol
10. Wash with 80 % alcohol
11. Wash with 96 % alcohol
12. Stain in Papanicolaou's stain OG6 solution 3 min
13. Wash with 96 % alcohol
14. Wash with 96 % alcohol
15. Stain in Papanicolaou's solution EA50 3 min
16. Dehydrate with 96 % alcohol
17. Dehydrate with 96 % alcohol
18. Dehydrate with absolute alcohol 5 min
19. Dehydrate with equal parts of absolute alcohol and xylene or xylene substitute.
20. Clear with xylene or xylene substitute.
21. Clear with xylene or xylene substitute 2 min
22. Mount with DePeX® or DPX mountant

Specimens for use in histology and cytology must be completely anhydrous prior to being mounted. Xylene should be added as a final stage in order to prevent turbidity brought about by solvents containing water.
To carry out the mounting process, drop approximately 0.5 ml mounting agent onto a horizontal slide using a glass rod. This fills the space between slide and coverglass. As soon as the specimen has been covered with a homogeneous solution, cover with a coverglass, taking care to avoid air bubbles.
Allow to harden over a period of 20-30 minutes in a horizontal position.

Result

The microscope used should meet the requirements of a medical diagnostic laboratory

Cytoplasm Cyanophilic (basophilic)	Blue-green
Cytoplasm Eosinophilic (acidophilic)	Pink
Cytoplasm Keratinised	Pink-orange
Erythrocytes	Red
Nuclei	Blue, dark violet,black
Microorganisms	Grey-blue
Trichomonas	Grey-green

Evaluate the result by comparing it to what would be the age related normal values

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Diagnostics

Diagnoses are only to be made by authorised and trained persons. Valid nomenclatures must be used.
Further tests must be selected and implemented according to recognised methods.

Storage

The staining solutions must be stored at +15°C to +25°C. After the first opening of the bottle the contents can be used up to the expiry date when stored at +15°C to +25°C.

Shelf life

The solutions must be used by the expiry date stated.
The bottles must be kept tightly closed at all times. Avoid warming of the solutions.

Auxiliary reagents

Cat. No	Description	Pack Size
36126	Microil Immersion Oil tropical grade	100 ml
36104	Microil Immersion Oil	100 ml, 500 ml
36102	Lenzol Immersion Oil Gurr	100 ml
36194	Fractoil Synthetic Immersion Oil	500 ml
28975	Xylene Mixture of Isomers Normapur Anal	1 l, 2,5 l, 5 l, 25 l
36125	DePeX® mounting medium	500 ml
36029	DPX mountant	100 ml, 500 ml

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Precautionary measures on health hazards
Effective measures must be taken to protect against infection in line with laboratory guidelines.

Physical Hazard classification
Please observe the hazard classification on the label and the information given in the safety data sheet.
The VWR safety data sheet is available on the Internet.

Instructions for environmental disposal
Used solutions and solutions that are past their shelf-life must be disposed of as special waste according to local disposal guidelines.VWR International can provide technical support for local disposal solutions.

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