65065-85
65066-85
65067-75
65067-85
65068-85
65069-85
7052X-75
7052X-85
7052X-86
7054X-85
7054X-86
7058X-75
7058X-85
7062X-75
7062X-85

Papanicolaou's Staining Procedure
Microscopy

Harleco® Hematoxylin Gill I
Harleco® Hematoxylin Gill II
Harleco® Hematoxylin Gill III
Harleco® Gill Modified EA
Harleco® Gill Modified OG
Harleco® Papanicolaou OG-6
Harleco® Papanicolaou EA-65
Harleco® Papanicolaou EA-36
Harleco® Papanicolaou EA-50

IVD In Vitro Diagnostic Medical Device

Intended Use

Harleco® Papanicolaou's Stains are used in combination for the cytological investigation of sample material of human origin.

Principle

The most used staining procedure for cytological specimens is the Papanicolaou's technique[1]. In the first staining step, cell nuclei are stained with a hematoxylin solution. This is followed by a bluing step in water to make the color permanent. The second staining step is cytoplasmic staining with an orange staining solution, which is used to demonstrate mature and keratinized cells. The target structures are stained orange in different intensities. In the third staining step the polychrome solution, a mixture of eosin, light green SF and Bismarck brown is used to demonstrate differentiated squamous epithelial cells.

Sample material

Cytological smears must be fixed immediately in 95% ethyl alcohol for a minimum of 15 minutes or fixed immediately with fixatives marketed in spray cans. These fixatives usually contain alcohol or ether alcohol with a small proportion of polyethylene or propylene glycol as a coating agent.

Reagent

Cat. No. 65065 — 4 L
Harleco® Hematoxylin Gill I

Cat. No. 65066 — 4 L
Harleco® Hematoxylin Gill II

Cat. No. 65067 — 1 L, 4 L
Harleco® Hematoxylin Gill III

Cat. No. 65068 — 4 L
Harleco® Gill Modified EA

Cat. No. 65069 4 L
Harleco® Gill Modified OG

Cat. No. 7052X 1 L, 4 L, 10 L
Harleco® Papanicolaou OG-6

Cat. No. 7054X 4 L, 10 L
Harleco® Papanicolaou EA-65

Cat. No. 7058X 1 L, 4 L
Harleco® Papanicolaou EA-36

Cat. No. 7062X 1 L, 4 L
Harleco® Papanicolaou EA-50

Also required:

Cat. No. 65348	Harleco® Alcohol, 95%	4 L
Cat. No. S5134	Scott's Tap Water Substitute Concentrate	6 x 100 mL
Cat. No. 64969	Harleco® Krystalon™ Mounting Medium	0.05 L, 0.5 L

Sample preparation

The sampling must be performed by qualified personnel. All samples must be clearly labeled. Suitable instruments must be used for collecting and preparing samples. Follow the manufacturer's instructions for application/use.

Modified Papanicolaou Staining Method

1. Fixation: 95% ethyl alcohol (See Sample Material)
    a. 15 minutes (minimum)
2. Hydration: Tap water x 2
    a. 10 dips each
3. Chromatin Staining: Hematoxylin Gill I (65065) or Hematoxylin Gill II (65066) or Hematoxylin Gill III (65067)
    a. 2 minutes
4. Rinse: Tap water x 2
    a. 10 dips each
5. Bluing: Scott's tap water substitute (S5134).
    a. Preparation of Scott's Tap Water Substitute: Mix 1 volume Scott's Tap Water Substitute Concentrate with 9 volumes deionized water.
    b. 1 minute
6. Rinse: Tap water x 2
    a. 10 dips each
7. Dehydration: 95 % ethyl alcohol x 2
    a. 10 dips each
8. Cytoplasmic Counterstaining: Gill Modified OG (65069) or Papanicolaou OG-6 (7052X)
    a. 1 minute
9. Rinse: 95% ethyl alcohol x 3
    a. 10 dips each
10. Cytoplasmic Counterstaining: Gill Modified EA (65068) or Papanicolaou Stain EA-65 (7054X) or Papanicolaou Stain EA-36 (7058X) or Papanicolaou Stain EA-50 (7062X)
    a. 4 to 10 minutes
11. Complete Dehydration: SDA-3A Alcohol x 3
    a. 10 dips each
12. Clearing: Xylene x 3
    a. 10 dips each
13. While still wet, mount using Krystalon™ (64969)
14. Examine the slide microscopically.

Results

Epithelial cells

- Nuclei should stain blue with clear, sharp details.
- Cytoplasm should stain with varying shades of pink, blue, yellow, green and gray.

Technical notes for Manual Staining Procedures

1. Experimentation and adjustment to staining times may be required to obtain optimal results and cell differentiation.
2. Best results are obtained when the following are observed:
    a. Slides are clean and free of grease and debris.
    b. Cytological smears are wet-fixed immediately after collection.
    c. A thin monolayer smear is preferred for optimal staining and display.
    d. Thick smears may take more stain and hinder microscopic examination.

Diagnostics

Diagnoses are to be made only by authorized and trained personnel. Valid nomenclature must be used. Further tests must be selected and implemented according to recognized methods. Suitable controls should be conducted with each application.

Storage

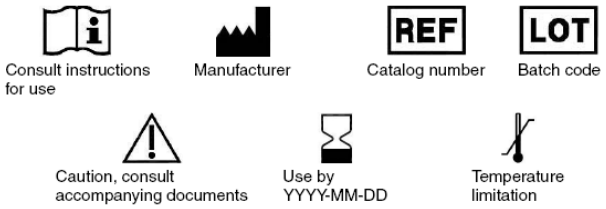
15-25 °C

Shelf-life

The Papanicolaou's Stains for microscopy can be used until the stated expiry on the packaging.

After first opening of the bottle, the contents can be used up to the stated expiry date when stored at 15-25 °C.

The bottles must always be kept tightly closed.



Additional instructions

For professional use only.

The application must be carried out by qualified personnel only.

National guidelines for work safety and quality assurance must be followed.

Microscopes equipped according to the standards must be used.

Protection against infection

Effective measures must be taken to protect against infection in line with laboratory guidelines.

Instructions for disposal

The package must be disposed of in accordance with the current disposal guidelines. Used solutions and solutions that are past their shelf-life must be disposed of as special waste in accordance with local guidelines.

Auxiliary reagents

Cat. No. 65348	Harleco® Alcohol, 95%	4 L
Cat. No. S5134	Scott's Tap Water Substitute Concentrate	6 x 100 mL
Cat. No. 64969	Harleco® Krystalon™ Mounting Medium	50 mL, 500 mL
Cat. No. 104699	Immersion oil for microscopy	100 mL, 500 mL

Hazard classification

Please observe the hazard classification printed on the label and the information given in the safety data sheet. The safety data sheet is available on the website and on request.

Literature

1. The Pap Smear, 1st Edition, Boon M.E., and Suurmeijer A.J.H. Coulomb Press Leyden 1991
2. Conn's Biological Stains: A Handbook of Dyes, Stains and Fluorochromes for Use in Biology and Medicine, 10th Edition, (ed. Horobin, R.W., and Kiernan, J.A.) Bios, 2002

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