IVD In Vitro Diagnostic Medical Device
For professional use only

Haematoxylin solutions

34037 Haematoxylin 1-hydrate Gurr
For microscopical staining (C.I. 75290)
35194 Haematoxylin Harris mercury free

Cat. No	Pack Type	Pack Size
340374T	Glass Bottle	25 g
340375U	Glass Bottle	100 g
351945S	Glass Bottle	1 l

Composition

Cat. No. 35194
C.I. 75290 5.3 g/l
$Al_2(SO_4)_3$ x 18 H_2O 34.4 g/l

Cat. No. 34037
C.I. 75290 95%

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 microsc ope
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). CLARKE 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

Application

Haematoxylins are mixed with trivalent positively charged metal salts and build up the so-called haematoxylin lakes, used for the selective staining of nuclei (DNA). Haematoxylin or better haematein builds up complex structures with the metal ions of the alums (Al, Cr or Fe), chelate rings. They are used in mild acid milieu and give the typical blue colour by the so-called blueing (= rinsing in tap water). This step fixes the colouration with the dye on the target structures.
Two methods can be distinguished. With the progressive method staining is carried out to the desired intensity, followed by the blueing step in tap water to make the colour permanent.
With the regressive method the material is over-stained and the excess of staining solution is removed by acid rinsing steps, followed by the blueing step to make the colour permanent.
The structures of nuclei are more differentiated and better visible by the regressive method.

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.

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Reagent

Cat. No	Description	Pack Size
35194	Harris hematoxylin soln.	1 l
34037	Hematoxylin (monohydrate) (C.I. 72590)	25 g, 100 g
10107	Ethanol 'absolute' 'AnalaR'	500 ml, 1 l, 2.5 l
21110	Aluminium Potassium Sulphate 12H_2O NP AR	1 kg
20104	Acetic Acid 100% Normapur	1 l, 2,5 l, 5 l, 20 l
	Aluminium sulphate 'AnalaR'	500 g
24041	Ethylene Glycol Normapur	1 l, 2,5 l, 5 l, 20 l
20728	Thymol Rectapur	250 g, 1 kg

Additional necessary

Cat. No	Description	Pack Size
35040	Papanicolaou's stain OG 6 Gurr	1 l
35169	Papanicolaou's EA50 (new formulation)	1 l

Preparation

1. Haematoxylin solution acc. to Harris
Dissolve 5 g haematoxylin Certistain® or haematoxylin crystalline in 50 ml ethanol under heating in a water bath, dissolve 100 g Aluminium potassium sulphate in 950 ml distilled water by stirring and heating.
Add the haematoxylin solution to the hot aluminium potassium sulphate solution under stirring and heat up to boiling, remove the solution from the heat. Add 370 mg sodium iodate under stirring and cool down in a water bath quickly. Add 4 ml acetic acid. Filter in bottles and close carefully. Filter before use.

2. Haematoxylin solution acc. to Gill
Dissolve 2 g haematoxylin, 0.2 g sodium iodate and 17.6 g aluminium sulphate (x18 H_2O) in a mixture of 250 g ethanediol and 730 ml distilled water. Add 20 ml acetic acid, stir for 1 hour at room temperature. Filter before use.

3. 0.1% aqueous hydrochloric acid solution
Fill up 27.5 ml HCl 1 N to 1 l with distilled water.

4. Sodium hydrogen carbonate solution 1.5%
Dissolve 15g $NaHCO_3$ in 1 l distilled water.

Procedure

Manual staining - progressive:

1. Wash with 96 % alcohol*.
2. Wash with 80 % alcohol*.
3. Wash with 70 % alcohol*.
4. Wash with 50 % alcohol*. *If Cytology spray fixative is used, steps 1 – 4 can be dropped.
5. Wash with distilled water
6. Stain in hematoxylin solution
   - Harris' hematoxylin solution 3 min
   - Hematoxylin solution acc. to Gill 2-5 min
7. Rinse under weak stream of tap water 3-5 min
8. Wash with 70 % alcohol
9. Wash with 80 % alcohol
10. Wash with 96 % alcohol
11. Stain in Papanicolaou's stain OG 6 Gurr solution for 3 min
12. Wash with 96 % alcohol
13. Wash with 96 % alcohol
14. Stain in Papanicolaou's EA50 (new formulation) for 3 min
15. Dehydrate with 96 % alcohol
16. Dehydrate with 96 % alcohol
17. Dehydrate with absolute alcohol for 5 min
18. Dehydrate with equal parts of absolute alcohol and xylene or xylene substitute
19. Clear with xylene or xylene substitute
20. Clear with xylene or xylene substitute for 2 min
21. 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.

Manual staining regressive:

1. Wash with 96 % alcohol*.
2. Wash with 80 % alcohol*.
3. Wash with 70 % alcohol*.
4. Wash with 50 % alcohol*. *If Cytology spray fixative is used, steps 1 – 4 can be dropped.
5. Wash with distilled water

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6. Stain in haematoxylin solution
   - Harris' haematoxylin solution 6 min
   - Haematoxylin solution acc. to Gill 5 min
7. Rinse in distilled water 10 sec
8. Rinse in HCl 0,1% 10 sec
9. Rinse in distilled water 10 sec
10. Rinse in sodium hydrogen solution 1.5% 1 min
11. Rinse under weak stream of tap water 3 min
12. Wash with 70 % alcohol
13. Wash with 80 % alcohol
14. Wash with 96 % alcohol.
15. Stain in Papanicolaou's stain OG 6 Gurr solution for 3 min
16. Wash with 96 % alcohol
17. Wash with 96 % alcohol
18. Stain in Papanicolaou's EA50 (new formulation) for 3 min
19. Dehydrate with 96 % alcohol
20. Dehydrate with 96 % alcohol
21. Dehydrate with absolute alcohol 5 min
22. Dehydrate with equal parts of absolute alcohol and xylene or xylene Substitute
23. Clear with xylene or xylene substitute
24. Clear with xylene or xylene substitute for 2 min
25. 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.

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Result

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

Staining with	**3b/EA 50**
Cytoplasm	
Cyanophilic (basophilic)	blue-green
Eosinophilic (acidophil)	pink
Keratinised	pink-orange
Erythrocytes	red
Nuclei	blue, black, dark violet
Microorganisms	grey-blue
Trichomonades	grey-green

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.
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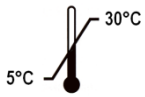
Diagnostics

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.



The staining dyes must be stored at +5°C to +30°C.The solutions must be used by the expiry date stated.

Shelf life

After the first opening of the bottle the solutions can be used up to the expiry date when stored at +15°C to +25°C and the dyes +5°C to +30°C.
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	500ml
36029	DPX mountant	100ml, 500ml

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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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