DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

EA50 solutions technical information
Technical card code 09-266-2
Product code 09-266-2
Stability of product properly conserved 24 month.
Pack 500-1000 ml or on request

Produce in Italy by:
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in case of emergency UE number	☎	112
in case of emergency UK number	☎	999
en cas d'urgence Suisse number	☎	145

Staining solutions and dyes for cytological cancer and cycle diagnosis

Principle. The most used staining procedure for cytological specimen is Papanicolaou 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 keratinized 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. 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, used for the selective staining of nuclei (DNA). Haematoxylin or better hematein 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 bluing (= 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 bluing step in tap water to make 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 bluing step to make colour permanent.

Orange G solution gives a pale, yellow-orange staining result with mature and keratinized squamous cells. Orange II solution gives an ore intense reddish staining result with mature and keratinized squamous cells. Polychrome solutions EA31 and EA50, are used for gynaecological material normally. Polychrome solutions EA65, are normally used for mucous rich, non gynaecological material. Papanicolaou solution gives red staining results while EA65 solution gives blue-green staining results. The structures of nuclei are more differentiated and better visible by the regressive method.

Sample material

Gynaecological and non-gynaecological specimens such as sputum, urine, FNAB, body effusions, lavages.

Fixation & Method. Wet fixation immediately with spray fixative DdFixx

Manual staining - progressive:
1. Wash with 96 % alcohol*
2. Wash with 80 % alcohol*
3. Wash with 70 % alcohol*
4. Wash with 50 % alcohol*

*If DdFixx is used, steps 1 - 4 can be dropped.

5. Wash with distilled water
6. Stain in haematoxylin solution
Haematoxylin solution; from 1 to 6 minutes, according to the type of stain used. Hematein solution 5 minutes
7. Rinse under weak stream of tap water 3-5 minutes
8. Wash with 70 % alcohol

9. Wash with 80 % alcohol
10. Wash with 96 % alcohol.
11. Stain in orange solution from 1 to 3 minutes, according to the type of stain used.
12. Wash with 96 % alcohol
13. Wash with 96 % alcohol
14. Stain in polychromatic or normal staining solution EA from 1 to 3 minutes, according to the type of stain used.
15. Dehydrate with 96 % alcohol
16. Dehydrate with 96 % alcohol
17. Dehydrate with absolute alcohol for 5 minutes
18. Dehydrate with equal parts of absolute alcohol and Greensolv or xylene. Mount with DdMount

1. Manual staining regressive:
2. Wash with 96 % alcohol*
3. Wash with 80 % alcohol*
4. Wash with 70 % alcohol*
5. Wash with 50 % alcohol*

*If DdFixx is used, steps 1 - 4 can be dropped.

6. Wash with distilled water
7. Stain in haematoxylin solution

Haematoxylin solution; from 1 to 6 minutes, according to the type of stain used. Hematein solution 5 minutes

8. Rinse in distilled water 10 second
9. Rinse in HCl 0,1% 10 second
10. Rinse in distilled water 10 second
11. Rinse in sodium hydrogene solution 1,5% 1 minutes
12. Rinse under weak stream of tap water 3 minutes
13. Wash with 70 % alcohol
14. Wash with 80 % alcohol
15. Wash with 96 % alcohol.
16. Stain in orange solution from 1 to 3 minutes, according to the type of stain used
17. Wash with 96 % alcohol
18. Wash with 96 % alcohol
19. Stain in polychromatic or normal staining solution EA from 1 to 3 minutes, according to the type of stain used.
20. Dehydrate with 96 % alcohol
21. Dehydrate with 96 % alcohol
22. Dehydrate with absolute alcohol 5 minutes
23. Dehydrate with equal parts of absolute alcohol
24. Clear with Greensolv or xylene
25. Clear with Greensolv or xylene. Mount with DdMount

Result

Staining with	EA 31	EA 50	EA 65	EA50 metachromatic
Cytoplasm				
Cyanophilic (basophilic)	intense green	blue-green	light red	pale blue green
Eosinophilic (acidophil)	pink	pink	red	pink
Keratinized	pink-orange	pink-orange	brownish red	pink-orange
Erythrocytes	red	red	brownish red	red
Nuclei	blue, black, dark violet			
Microorganisms	grey-blue			
Trichomonades	grey-green			

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Performance characteristics

The chromatin must appear between blue and black-blue, while the nucleoli should be clearly visible. The cytoplasmic staining with Gill 2 haematoxylin solutions are usually pale or absent, so the acid differentiation might not be necessary.

* Times vary according to age, types of solutions, thickness of sections, et. When Gill (code 09-178) modified solution is used, get the best result, staining time (maximum 1-5 minutes), for best change in color, wash quickly in tap water, and then in Scott acidulated solution, (code 00-136). For sections fixed in Bouin, we recommend the use of haematoxylin modified acid AB (code 09-183). Please note the alcoholic loses eosin stain with the use, of the days are stretched over time colouring. If you are using purified eosin, check the time, and possibly diluted in ethyl alcohol 96 ° C, if the cytoplasmic staining was too strong. Before use, filter the following solutions; alcoholic eosin, eosin phloxine; Harris haematoxylin, Gill's haematoxylin. The acidified aqueous solution of eosin is prepared by slowly adding glacial acetic acid.

Technical note
Follow normal precautions for laboratory reagents. Dispose of waste according to regulations at the local, regional or national level. Refer to Data Sheet Material Safety Data for updated information on risks, hazards and safety associated with the use of these products.

Risk and Safety Statements outside the EU.
The eosin solution in alcohol is flammable and harmful. Harmful by inhalation, in contact with skin or if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with the skin or by ingestion. Irritating to eyes, respiratory system and skin. Keep away from sources of ignition - No smoking. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible). Target organs: eyes and nerves. Eosin in aqueous solution. Caution: substance not yet fully tested.
Avoid contact and inhalation of the solution of Harris haematoxylin. Organs: heart and nerves. Solutions based haemalum are harmful. Harmful if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical attention. Wear suitable protective clothing. Organs affected: liver and kidneys. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible).

Risk and Safety Statements (U.E.)
The eosin solution in alcohol is highly flammable and harmful. Highly flammable. Harmful by inhalation, in contact with skin or if swallowed. Harmful: possible risk of irreversible effects through inhalation, in contact with the skin or by ingestion. Keep away from sources of ignition - No smoking. Wear suitable protective clothing and gloves. In case of accident or if you feel unwell, seek medical attention immediately (show the label where possible). Eosin in aqueous solution. Caution: Substance not yet fully tested. Solution of haemalum. Do not breathe vapors. Avoid contact with skin and eyes. Gill haematoxylin Solutions are harmful. Harmful if swallowed. Irritating to eyes, respiratory system and skin. In case of contact with eyes, rinse immediately with plenty of water and seek medical attention. Wear suitable protective clothing.

The microscope used should meet the requirements of a medical diagnostic laboratory. Carefully follow the instructions for the fixative. If an automated tool was used for staining, follow the instructions of the equipment and software. Remove surplus immersion oil before storing. Sample preparation. All samples must be treated according to the technology. All samples must be marked so as to be easily identified. Tools should be used for sampling and sample preparation, which must be observed strictly to manufacturer's instructions about the application and instructions.

Diagnostics
The diagnosis should be performed only by authorized and trained persons.
Valid nomenclatures must be used. Further tests must be selected and implemented according to recognized methods.
Conservation. The staining solution should be stored at a temperature between +15°C to +25°C, the dye at +5°C to +30 ° C. The solution and dyes must be used before the expiration date.

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Stability. A first opening the bottle, the dye solution and the dyes are stable until the expiration date when stored at a temperature of +15°C, respectively, to +25°C and +5°C and 30°C. Always keep the bottles tightly closed.
Instructions for use. To avoid errors, the staining process must be performed by qualified personnel.

For professional use only.
Must observe the National guidelines for work safety and quality assurance.
Microscopes are used according to the standard.

Protection against infection
Must be taken with laboratory guidelines for the protection against infection.
Instructions for disposal

The solutions used and those have expired must be disposed of as special waste according to local regulations regarding disposal of waste.

Endnotes
1 The timing suggested in the leaflet are approximate and may vary according to your specific needs. If they are used intensively, for staining solutions may lose their dyes, so it is necessary to extend the time of staining solutions, or replace with new products.
2. Include positive control slides in each session.
3. Some hydraulic systems deliver acidic water, unsuitable for use for the part of the procedure for the blue coloration. If tap water is acidic, instead using a dilute alkaline solution, for example, water buffered by Scott.
4. The presence of purple or red-brown nuclei a blue color indicates unsatisfactory.
5. If you over-eosin staining, nuclear staining may be masked. If done correctly, with eosin staining shows a three-tone effect. To increase the differentiation of eosin, extend the time of immersion in alcohol, or use a first alcohol with higher water content. You can adjust the times of immersion in alcohol to obtain an adequate eosin staining.
6. We do not recommend the addition of stock solution in the working solutions of haematoxylin and eosin.
7. Avoid excessive drag (carryover) of water solutions in alcoholic eosin.
8. The data generated by this procedure are to be used only to support the diagnosis and should be evaluated in conjunction with other tests and diagnostic data