DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Methylene blue polychrome solution technical information
Technical card code 09-122
Product code 09-122
Pack 500 ml or on request
Stability of product properly conserved in a dark place at 15-25°C 24 months.

Produce in Italy by:
DDKItalia S.r.l
Via Marche 19-27029 Vigevano (I)
info@ddkitalia.com ● www.ddkitalia.com

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

Principle

Haematological methods
The typical colour of cell nuclei, namely purple, is due to molecular interaction between eosin y and the methylene blue azure B-DNA complex. The staining result can be influenced by several factors as pH of the solutions and buffer solution, buffer substances, fixation, staining time. The haematological staining technique is used for visualisation of blood parasites protozoans in blood. The nuclei of blood parasites protozoans appear red under the microscope.

Bacteriological methods

Mycobacteria are difficult to stain because of the high proportion of lipid and wax in their cell walls. Up to now, in order to carry out the classical Ziehl-Neelsen staining, the test material has to be heated with carbol fuchsin solution to produce the mycolic acid fuchsin compound. Once stained, acid fast mycobacteria keep their colouring even after treatment with strong decolourizing solutions as HCl-ethanol. They remain red after counterstaining with methylene blue, whereas the microorganisms susceptible to acid take on the blue.

Sample material. Haematology. Air-dried blood smears

Bacteriology

Heat-fixed smears of sputum, FNAB, lavages, imprints, body fluids, exudates, pus, liquid and solid cultures, histological sections.
Bacteriology
1. Fixation. Fixation is carried out over the flame of a Bunsen burner (2-3 times, avoiding excessive heating).
It is also possible to fix the smears in an oven at 100-110 °C for 20 minute.
2. Löffler's methylene blue solution

Staining procedure.

Haematology.
Fixation is carried out in the first staining step with undiluted May-Grünwald solution

Staining rack

1. Onto each fresh, dried film, pipette just enough May-Grünwald solution to cover the blood film (usually 10 drops or more) and let react for 3 minute.
2. Add an equal amount of distilled water, mix and stain for 1 minute.
3. Pour off fluid and without washing add about 10 drops of diluted buffered Giemsa solution, stain for 5 - 60 minute (try first for 10 -15 minute).
4. Rinse with buffer solution.
5. Dry and examine under the microscope.

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Bacteriology

Staining rack
1. Flood specimens completely with Tb-color modified carbol-fuchsin solution. Carefully heat 3 times from below with a bunsen burner to steaming and keep hot for 5 minute. Do not allow the stain to boil.
2. Wash with tap water until no further colour is given off.
3. Cover completely with Tb-color modified decolourizing solution and, depending on the thickness of the specimen, allow to stand for 15 – 30 second.
4. Wash immediately with tap water.
5. Counter stain by flooding for 30 sec in Tb-color modified methylene blue solution or for 1 min with a diluted solution (dilution 1:10 (1+9) with distilled water)
6. Wash well with tap water.
7. Dry
Allow the specimens to dry and, if necessary, mount with DdMount
Dehydrate histological specimens (ascending alcohol series) and mount with DdMount.

Result

Haematology	
Nuclei	red to violet
Lymphocytes	plasma blue
	azure granules purple to red
Monocytes	plasma dove-blue
Neutrophilic granulocytes granules	light violet
Eosinophilic granulocytes granules	red to grey-blue
Basophilic granulocytes granules	dark violet
Thrombocytes	violet
Erythrocytes	red
Blood parasites nuclei	bright red

Bacteriology

Mycobacteria	red
Background	light blue

A positive finding is reported as "acid fast bacteria detected" and a negative finding is reported as "acid fast bacteria not detected". It is not possible to state whether there are tuberculosis bacteria or other "atypical" bacteria. It is also impossible to state whether these mycobacteria are still capable of reproduction or are already dead. When acid-fast bacteria are found in the material examined, further investigations in a special laboratory are indicated.

* Notes. Distilled water or tap water can be used for rinsing and moisturizing. Always check the pH of your tap water and chlorine levels before proceeding with any type of biological tissue and stain.

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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 a 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