DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Mycobact-Fluo cold fluorescence staining of mycobacterium technical information
Security card code 12-100
Product code 12-100
Pack 1kit. Number of tests 100 or on request
Stability of product properly conserved at 15-20°C 24 months

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

En cas d'urgence, contactez votre unité de poison contre le plus proche	UE	☎	112
En cas d'urgence, contactez votre unité de poison contre le plus proche	Suisse	☎	145

Principle

Auramine staining. This technique, has the same principle of Ziehl-Neelsen stain, but using a fluorophore (or auraminarodamina Auramine O), fluorescence in place of basic fuchsin phenolized use of which binds directly to lipids wall with physical and chemical mechanisms. Instead of methylene blue is used potassium permanganate which serves to mitigate any background fluorescence.

General recommendations for collecting samples. Medical personnel must treat as potentially infectious materials, must take their personal protective equipment (PPE). For proper processing the samples must be collected and transported to the laboratory by observing the following precautions: use disposable plastic sterile, waterproof, with screw cap. Avoid using buffer. Do not use fixatives or preservatives.

Method

Cover the slide with Auramine solution (1), stain for 15 minutes without heating.
Wash with running water gently to avoid the violent jet, you should use a plastic squeeze bottle with distilled water.
Discard the remaining water off the slide by draining or by shaking slightly.
Decolorize with acid-alcohol solution (2) for 2-3 minutes.
Wash with tap water or distilled accurately. Drain.
Stain with potassium permanganate for no longer than 2 minutes solution (3).
If allowed to act longer, potassium permanganate can bind auramine, thus masking the presence of acid-alcohol resistant bacilli.
Wash gently with tap water.
Remove residual water by shaking slightly the slide.
Air dry and observe within 24 hours a fluorescence microscope with 40x lens.

Result.

The fluorescent mycobacterium appears in bright yellow with dark background.

Reagents:

Auramine solution	30 ml
Acid-alcohol solution	30 ml
Potassium permanganate	30 ml

Preparation of the smear.

Using slides, well-scoured, appropriately marked with the identifying data of the sample. Prepare two slides per sample, and after decontamination. Transfer approximately 50 µl of material on the slide surface using a loop or a pipette. Distribute the material uniformly over an area of about 2x1 cm, taking care that the preparation is not too thick that hide the bacilli. Important is the choice of fragments of material to be spread on the glass: purulent sputum should be collected and possibly caseous particles.
For the fluid and bronchial aspirate is preferable to limit the distribution area of the material and use its "layering" that you run the loop stretching with a drop on the slide, waiting to dry and then repeating the process with other 2 - 3 time.
Air dry.
Fix slides 3-4 times not more whit the blue flame of a Bunsen burner.

DDK Italia

IVD dispositivo medico-diagnostico in vitro CE

Mycobact-Fluo cold fluorescence staining of mycobacterium technical information
Security card code 12-100
Product code 12-100

* Technical note: staining time 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.

* 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 hemallum 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 hemallum. 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.

DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Mycobact-Fluo cold fluorescence staining of mycobacterium technical information
Security card code 12-100
Product code 12-100

* Conservation. The staining solution should be stored at a temperature between +15°C to 20°C, the dye at +5°C to 30° C. Store at 4-6 °C all kit containing silver solutions and Schiff reagents. The solution and dyes must be used before the expiration date. Stability. After first opening the bottle, the dye solution and the dyes are stable until the expiration date when stored at the temperature requested. 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 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