DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Gram for bacteria technical information
Technical card code 14-111
Product code 14-111
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

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

Application. To differentiate between Gram-positive and Gram-negative bacteria in tissue sections and smears.

Principle

Gram staining is the most important method to differentiate bacteria species. Two dyes are used one after the other: crystal violet and fuchsine. Crystal violet solution precipitates through oxidation with a Lugol solution. The deriving complex attaches to bacteria cell walls with bonds of varying nature and intensity. The differentiating solution removes the crystal violet-iodine complex from the walls of some bacteria, but it does not act on others. These retain the primary dye and are called Gram-positive. Decolorized bacteria are then counterstained with a red dye; they are called Gram-negative. Gram-positive bacteria's capacity to retain the dye-Lugol iodine complex is usually ascribed to the bond which develops between the complex and a molecule only Gram-positive possess, namely magnesium ribonucleate.

Method

1) Bring section to distilled water.
2) Pour the content of bottle (1) in a coplin jar, introduce the slide and incubate at 56-58°C for 15 minutes; pour back the solution in bottle (1) filtering through filter paper. 3) Wash in distilled water.
4) Put on the section 10 drops of reagent (2), leave to act 3 minutes.
5) Drain the slide without washing and put on the section 10 drops of reagent (3), leave to act 3 minutes.
6) Wash in distilled water and dry the slide in filter paper then in the air for 10 minutes.
7) Pour the content of bottle (4) in a coplin jar: shake the slide for 1 minute; pour back the solution in bottle (4) filtering through filter paper.
8) Repeat step 7 with reagent (5).
9) Clear in xylene. Mount with DdMount

Results

Gram-positive bacteria:	blue
Gram-negative bacteria:	red
Nuclei:	red

Reagents

1 - Phloxine B solution	2x30 ml
2 - Crystal violet solution	30 ml
3 - Lugol solution	30 ml
4 - Acid differentiator	30 ml
5 - Acid differentiator	30 ml

* 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.

DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Gram for bacteria technical information
Technical card code 14-111
Product code 14-111

The acidified aqueous solution of eosin is prepared by slowly adding glacial acetic acid.

* 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.

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