DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Luxol fast blue (Klüver Barrera) technical information
Technical card code 14-115
Product code 14-1015
Pack 1kit. Number of tests 100 or on request
Stability of product properly conserved at 15-20°C 24 months

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	☏	145

Application. To show myelin and phospholipids in histological sections.

Principle

Luxol fast blue dye is a derivative of tetrabenzotetrazo-porphyrin. Kluver has demonstrated porphyrins have a selective affinity for myelin (see references). Luxol fast blue's affinity for central nervous system is usually ascribed to the bonds it forms with phospholipidic structures such as lecithin and sphingomyelin.

Method

1) Deparaffinize and bring section to ethanol 95°.
2) Prepare the incubation box by adding some drops of distilled water on filter paper in petri dish and lay down the slide; put on the slide 10 drops of reagent (1), close the incubation box and incubate at 56°C overnight in oven.
3) Extract the slide from oven and wash it with ethanol 95° (crystalline residues of reagent (1) should melt).
4) Wash in distilled water.
5) Put on the section 10 drops of reagent (2) leave to act 30 seconds.
6) Differentiate in ethanol 70° until myelinic fibers become blue on colourless background (Sometimes differentiation can be difficult; repeat the step 5 for 30 seconds and put the slide again in ethanol 70°).
7) Wash well in distilled water (at least 2 times).
8) Prepare the incubation box again and introduce the slide; put on the section 10 drops of reagent (3) and 5 drops of reagent(4), close the incubate box and incubate for 20 minutes at 56°C in oven.
9) Differentiate in ethanol 95° until Nissl substance results pale pink.
10) Dehydrate in absolute ethanol.
11) Clear in xilene
12) Mount with DdMount.

Results

Myelin:	turquoise blue
Neurons and glial nuclei:	pink to violet
Nissl substance:	pale pink

Reagents

1 - Luxol fast blue alcoholic solution	30 ml
2 - Basic differentiating buffer	30 ml
3 - Cresyl violet aqueous solution	30 ml
4 - Acid activation buffer	30 ml

* Technical's 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.

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* 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