DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Masson’s trichrome light green technical information
Technical card code 12-108
Product code 12-108
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

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Sections

Bouin's fluid or formal sublimate fixation are preferred.
Zenker and Helly are satisfactory. Formalin variants are adequate.
If formalin fixation is used, staining can be improved by secondary fixation of cut sections using Bouin's fluid.
Paraffin sections at 5 micron will be satisfactory.

Method

Take slides to distilled water
Place slides in Bouin for 60 minutes at 56°C if the sections were previously treated with formalin.
Leave them to cool for 10 minutes.
Wash in running tap water until sections are clean.
Wash with distilled water.
Place on sections Waigert haematoxylin A+B for 10 minutes (to be reconstituted in equal parts).
Wash in tap water for 10 minutes.
Wash with distilled water.
Place the sections in Biebrich scarlet - acid fuchsin solution for 15 minutes.
Wash with distilled water.
Differentiate in acid phosphotungstic for 10 - 15 minutes. Check that collagen is not red.
Repeat the fuchsin differentiation if has held the red.
Counterstain the green light (from acidifying, acidification is very subjective), for 1 to 2 minutes.
Wash quickly in distilled water.
Dehydrate, go to alcohol and xylene
Mount with DdMount

Results

Nuclei:	black
Muscle, cytoplasm, keratine	red
Collagen:	green

Reagenti

Waigert haematoxylin (A & B)	2x30 ml
Biebrich scarlet	2x30 ml
Phosphotungstic acid	2x30 ml
Green light	2x30 ml

Notes

Since Masson gave details of his original method, there have been large numbers of variations published. One common variant is Lillie's, a method often erroneously called "Masson's" trichrome. These variants differ in the specifics of the dyes used, the concentration of the dyes and polyacid and the times for which they are applied.
The times given here should be considered a guide, although they will generally be found satisfactory if the procedure is followed completely, including refixation of sections in Bouin's fluid. Refixing the sections in Bouin's fluid intensifies the colours and increases the contrast between the tissue components. It is sometimes incorrectly referred to as mordanting, but it is simply a form of secondary fixation of sections and has no real mordanting

effect. It should be noted that primary fixation in Bouin's fluid is recommended for this method. The dyes in solution A stain the muscle, fibrin, cytoplasm and erythrocytes and the solution is sometimes referred to as the *plasma* stain. Acid fuchsin and Xylidine ponceau are the dyes originally recommended. Many other red acid dyes have been suggested. Biebrich scarlet, for instance, being used by Lillie. Solution C is sometimes called the *fibre* stain. It is used to colour collagen. Some other materials will also be coloured, but the method is not used to demonstrate them. Light green SF yellowish is commonly used, but Fast green FCF gives a more emerald green. This is preferred by some microscopists. Although this latter dye is considered less likely to fade, it is not as commonly used. Aniline blue, or either of its constituents, Methyl blue, or Water blue may be substituted alone or in combination if blue stained collagen is preferred to green.

* 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.

* 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.

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* 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 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