

Nile blue sulphate method for acidic and neutral lipids (for research purpose only) technical information

Technical card code 14-150

Product code 14-150

Pack 1kit. Number of tests 50 or on request

Stability of product properly conserved at 4°C 6 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

Principle

Nile blue sulphate serves as a preliminary test to indicate the distribution of neutral versus acid lipids. Nile blue sulphate comprises two components; a red oxazone which dissolves in neutral lipids and a blue oxazine which is basic and reacts with phospholipids and free fatty acids.

Fixation and sections

Section must be fixed in NBF acetate buffer.

Cryostat sections post-fixed for one hour in acetate formalin. Short fixed frozen sections.

Procedure

1. Cut frozen sections and fix for 1 hour in acetate formalin.
2. Stain in Nile Blue Sulphate at 60°C for 30 minutes.
3. Differentiate in 1% acetic acid for 1 - 2 minutes.
4. Wash well
5. Counter stain nuclei with methyl green 5 minutes.
6. Wash well. Mount with D-Mount jelly.

Results

Unsaturated hydrophobic lipids:	pink
Free fatty acids:	pink to blue
Phospholipids:	blue

Reagent

Blue Nile sulphate	30 ml
Acetic acid 1%	30 ml
Methyl green	30 ml

References

p 170 & 223 Bancroft JD & Stevens, Theory & Practice of Histological Techniques, 2nd Ed. p 111 Cook H C, Manual of Histological Demonstration Techniques. p 363 Culling CFA, Handbook of Histopathological and Histochemical Techniques.

* 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.

* 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

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