

AgNor kit technical information

Security card code 14-100

Products code 14-100

Number of test 12 assay (up to four slides for each assay)

Storage temperature 4°C in darkness place

Stability of reagents properly conserved 12 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 argentaffine proteins (100 KD), which are present in nucleolar organizer region (NOR), in smears and sections embedded in paraffin.

Principle

Nucleolar proteine 100 KD acts with RNA polymerase I to form AgNOR that is proteins bound to NOR which can be showed by silver impregnation.

Argyrophilic reaction is due to the intrinsic capacity of 100 KD to act as a reducing substance on silver ions in the solution, binding them in the reduced form of metallic silver.

Just before use, the silver nitrate solution is mixed with positively charged gelatine aqueous solution that favours the contact between reducing groups of nucleolar proteins and silver ions.

Warning

Always use excellent distilled water to wash the slides. Do not use poly-lysine coated glass slides. Avoid the use of metallic object (tweezers, slide racks etc.). After mounting, keep the slides in a dark place.

If reagent (1), stored at lower temperature than advised one, results in gel form can be fluidified bringing bottle under warm tap water.

Method

- 1) Bring sections to distilled water.
- 2) Prepare the working-solution: place the slide container in the polystyrene support. Introduce in the container the whole content of bottle (1) together with the whole content of bottle (2), and ten drop of solution (3). Stir briefly with a glass stick previously washed in distilled water.
- 3) Introduce the slide in the solution and incubate in the darkness for 60 minutes at 37 °C for.
- 4) Wash carefully in 3 changes of distilled water.
- 5) Counter stain with Mayer haemallum for five minutes.
- 6) Wash in distilled water.
- 7) Dehydrate through ascending alcohols; clear in xylene and mount with DdMount.

Results

AgNOR, argentaffin granules: black

Reagents

1. Gelatin aqueous solution	2x16 ml
2. Silver nitrate concentrate solution	2x 24 ml
3. Fixing solution	1x30 ml
4. Mayer haemalum	30 ml

References

Ghimenton C, Sbabo L. *AgNOR: metodica e possibili applicazioni in patologia*. Bio informazioni 1992; 1: 65-73. Ploton D, Menager M, Jeannesson P, Himber G, Pigeon F, Adnett JJ. Improvement in the staining and in the visualisation of the argyrophilic proteins of the nucleolar organizer region at the optical level. Histochem

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* 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.

* 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 haemallum 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 haemallum. Do not breathe vapours. 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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