DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Von Kossa method for calcium technical information
Technical card code 14-131
Product code 14-131
Pack 1kit. Number of tests 100 or on request
Stability of product properly conserved at 4°C 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 demonstrate deposits of calcium ions in tissue sections.

Principle

This method is based on a substitution reaction. Tissue is treated with a silver nitrate solution; cationic silver replaces calcium in the original salt thus forming a silver salt which can be showed by a reduction to metallic silver. Reaction is as follows:

$CaCO_3 + AgNO_3 = Ag_2CO_3 + Ca(NO_3)_2$

$Ag_2CO_3 + 2H_+ = 2Ag + H_2O + CO_2$

Calcium salts liable to react with silver nitrate are: carbonate, phosphate, oxalate, sulfate, urate, chloride, sulfocyanide. Since calcium is present in mammalian tissue essentially in the form of calcium carbonate and calcium phosphate, this method is suitable to point it out. To prevent false results caused by the presence of uric acid and urates in the tissue, these salts have been solubilised in a lithium carbonate saturated solution.

Method

1) Bring section to distilled water.
2) Put on the section 10 drops of reagent (1), leave to act 10 minutes.
3) Rinse well in distilled water.
4) Put on the section 10 drops of reagent (2), leave to act in the dark 1 hour.
5) Rinse well in distilled water.
6) Put on the section 5 drops of distilled water and add 10 drops of reagent (3), leave to act 5 minutes (Until silver salts become black).
7) Rinse in distilled water.
8) Put on the section 10 drops of reagent (4), leave to act 5 minutes.
9) Rinse in distilled water.
10) Put on the section 10 drops of reagent (5), leave to act 5 minutes.
11) Rinse in distilled water and
12) Dehydrate through ascending alcohols.
13) Clear in xylene.
14) Mount with DdMount.

Results

Sites calcium deposits:	black
Nuclei:	red

Reagents

1 - Lithium carbonate saturated solution	30 ml
2 - Silver nitrate solution	30 ml
3 - Reducing solution	30 ml
4 - Sodium sulphite solution	30 ml
5 - Mayer's Carmalum	30 ml

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* 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.

* 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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