DDK Italia

IVD dispositivo medico-diagnostico in vitro

CE

Sirius red E.P.S.R. technical information
Technical card code 14-145
Product code 14-145
Pack 1kit. Number of tests 100 or on request
Stability of product properly conserved at 15-20°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

Description. It's one of the best understood techniques of collagen histochemistry. Technical details follow, and are followed by some comments and a few references. You should come to grips with the theory, advantages and limitations of this method before using it on a large scale. Picro-sirius red method (after Puchtler et al., 1973; Junqueira et al., 1979). Step 4 is an addition that prevents the loss of dye that happens if the stained sections are washed in water.
Fixation. Fixation is not critical, the method is most frequently used on paraffin sections of objects fixed adequately (at least 24 hours but ideally 1 or 2 weeks) in a neutral buffered formaldehyde solution. This protocol has not been tested on frozen sections.

Method

1. De-wax and hydrate paraffin sections.
2. Stain nuclei with Weigert's haematoxylin for 8 minutes, and then wash the slides for 10 minutes in running tap water).
3. Stain in picro-sirius red for one hour (This gives near-equilibrium staining, which does not increase with longer times. Shorter times should not be used, even if the colors look ok.)
4. Wash in two changes of acidified water (not included).
5. Physically remove most of the water from the slides by vigorous shaking.
6. Dehydrate in three changes of 100% ethanol. Clear in xylene and mount with DdMount.

Results

In bright-field microscopy	collagen is red on a pale yellow background.
Nuclei, if stained	are ideally black but may often be grey or brown.

The long time in picro-sirius red causes appreciable de-staining of the nuclei. This is not a problem with traditional van Gieson or with picro-aniline blue, with their one-minute staining times.)

When examined through crossed polars the larger collagen fibers are bright yellow or orange, and the thinner ones, including reticular fibers, are green. According to Junqueira et al. (1979) the birefringence is highly specific for collagen. A few materials, including Type 4 collagen in basement membranes, keratohyaline granules and some types of mucus, are stained red but are not birefringent. It is necessary to rotate the slide in order to see all the fibres, because in any single orientation the birefringence of some fibres will be extinguished. This minor inconvenience can be circumvented by equipping the microscope for use with circularly rather than plane polarized light (Whittaker et al., 1994; Whittaker, 1995), but then you don't get a completely black background.

Reagents

1 - Weigert's haematoxylin	2x30 ml
2 - Sirius red F3B solution	50 ml

Comments and References. Although this method is technically very easy, it is important for the person doing it and (if it's someone else) the person using the stained slides, to know what it does and how it works. Even without a polarizing microscope, picro-sirius red shows things like reticular fibres and the basal laminae of cerebral capillaries, which are missed by van Gieson and may be obscured by masses of other stained details in trichrome methods (Mallory, Masson, Heidenhain etc).

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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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