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FAQ: Picrosirius Red Stain Kit

BACKGROUND

Yellow picric acid dye is small, anionic and slightly hydrophobic and Sirius Red is a red acid dye that is large and hydrophilic, combined with a metal complex cationic dye is the basis for the mechanism that stains collagen fibers and associated tissues. Initially, nuclei and cytoplasm are stained and the metal complex dye like Weigert's hematoxylin is stable to withstand the acid dyes in subsequent steps. If necessary, staining may be differentiated in acid alcohol. The second staining step, stains collagen red, cytoplasm and other protein rich material being stained different shades of yellow. The staining patterns are controlled at a rate by the size of the dye molecule which is large and slower to diffuse permeable sites: the collagen fibers. Fixatives that increase staining rates result in a redder section, whereas thick sections stain more yellow than thin sections. Selectivity is influenced by a variety of factors like time, temperature, fixation, thickness of sections and pH.

WEIGERT'S HEMATOXYLIN

PROCEDURE

1. De-wax and hydrate paraffin sections.
2. Stain nuclei with Weigert's hematoxylin for 8-10 minutes, wash slides for 10 minutes in running tap water.
3. Stain in Picrosirius Red for one hour
4. Wash in two changes of acidified water.
5. Physically remove most of the water from the slides by shaking and blotting sections.
6. Dehydrate in three changes of 100% ethanol very quickly.
7. Clear in xylene and mount in a resinous medium.

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 1-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 fibers, because in any single orientation the birefringence of some fibers 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.

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, Picrosirius Red shows things like reticular fibers and the basal lamina 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).

If you are using only polarized light it does not matter if you lose the "yellow background" of picric acid staining. If you use Picrosirius Red as a "better" van Gieson and want to keep the yellow cytoplasm, be hasty with the dehydrating even more so than with the original van Gieson method. Nobody should do (or order to be done) a Picrosirius Red stain without reading at least one of the first two items listed below.

1. Junqueira LCU, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. Histochem J 1979; 11, 447-455
2. Puchtler H, Waldrop FS, Valentine LS. Polarization microscopic studies of connective tissue stained with picro-sirius red FBA. Beitr Path 1973; 150, 174-187
3. Whittaker P. Polarized light microscopy in biomedical research. Microscopy and Analysis 1995; 44, 15-17
4. Whittaker P, Kloner RA, Boughner DR, Pickering JG. Quantitative assessment of myocardial collagen with picrosirius red staining and circularly polarized light. Basic Research in Cardiology 1994; 89, 397-410
5. Kiernan. J.A., (1999) Histological and Histochemical Methods: Theory and Practice, Ed. 3 Butterworth Heinemann, Oxford, UK.

Alternate Protocol: using acidifed water (acidulated water); **Acidified Water:** Add 5ml acetic acid (glacial) to 1 liter of water (tap or distilled).

Modifications of the procedure may be carried out with celloidin, cryophilic resin and paraffin sections and although there are fixation effects on the color balance of staining, fixation is not critical.

TIPS FOR SUCCESSFUL STAINING

Keep suitable blocks that stain well as a control and for efficacy of procedures.

If nuclei fail to stain, make sure that an iron hematoxylin or Celestine Blue was used. If an aluminum hematoxylin was used, switch to iron hematoxylin or Celestine Blue.

Check under the microscope to ensure that nuclear staining is intense before proceeding to place slides in Picrosirius Red Stain, since the picric acid acts as a differentiating agent.

When unexpected structures stain, it is a fixative effect. The staining has changed because of a change that has occurred in the fixative.

If sections are too red or too yellow than expected results, check the fixative. A coagulant fixative produces redder tones and a crosslinking fixative produces yellow tones.

If cytoplasm stains red, the Picrosirius Red Stain has hydrolyzed under acid conditions and higher temperatures, facilitaties in very warm climates should be aware of this phenomenon.

If thick sections are cut for neuroanatomical work and are much more yellow than anticipated, this is section thickness artifact. Try longer staining times or stain in a heated dye-bath.

If sections become redder during removal of excess stain and dehydration, the small picric acid dye may have been selectively extracted by the rinse (if used) or by the dehydration alcohols if water content is high in the alcohols, thus leaving a large hydrophilic red acid dye. Replace any aqueous step by blotting, rinsing in alcohol and or shorter dehydration times.

Specimens embedded in hydrophilic resins may show no red staining in regions that are expected to be collagen rich. The resin may be excluding the larger red dye. Try longer staining times, adding a little ethanol to the staining bath as a plasticizer or staining in a heated dye bath.

If your sections have faded or become yellow upon storage, keep the red acid dyes out of direct sunlight. Sunlight fades red acid dyes.

You can always increase the amount of cross-linker in the resin mix if you are experiencing wrinkling of hydrophilic resin embedding.

Baker (1958)
Horobin and Flemming (1988)
Prento (1993)
Shoobridge (1983)

ORDERING INFORMATION

Cat. #	Description
24901	Picrosirius Red Stain Kit

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