

Picrosirius Red Stain Kit

Catalog Number: 24901

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 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.

Picrosirius red method is used to stain collagen I and III. The stain will quantify the amount of collagen in a given area of myocardial tissue, i.e. the collagen area fraction. Picrosirius Red Stain binds specifically to collagen fibrils of varying diameter that is used to distinguish collagen type I from collagen type III. Collagenous structures of the mandible stained brilliant red. Dentinal tubules, Sharpey's fibers and other structures not easily seen in sections stained with hematoxylin and eosin alone were seen clearly after this procedure. Under polarized light collagen fibers could be specifically identified and their orientation determined. Picrosirius Red Stain hematoxylin is recommended for examination of normal or pathologic dental specimens.

CONTENTS

Solution A - Phosphomolybdic Acid
Solution B - Picrosirius Red F3BA Stain
Solution C - 0.1 N Hydrochloride Acid

FIXATION

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 solution.

PROCEDURE (PARAFFIN EMBEDDED TISSUE)

1. Deparaffinize and hydrate to DI water.
2. Stain in Weigert's Hematoxylin for 8 minutes (optional) if Weigert's hematoxylin is not used, go directly to step four.
3. Rinse well in DI water.
4. Place in Solution A for 2 minutes.
5. Rinse in DI water.
6. Place in Solution B for 60 minutes.
7. Place in Solution C for 2 minutes.
8. 70% Ethanol for 45 seconds.
9. Dehydrate, clear and mount.

PROCEDURE (FROZEN TISSUE¹)

1. Fix fresh frozen tissue in 10% formalin for 2 to 5 minutes.
2. Rinse in DI water.
3. Stain in Picrosirius Red (Solution B) for 90 minutes.
4. Rinse in HCl (Solution C) for 1 minute (2 times).
5. Rinse in DI water.
6. Dehydrate in 70% EtOH for 30 seconds.
7. Visualize under polarized light.

RESULTS

Stains fibrillar type I and type III collagen.

Collagen	Red
Type I	Yellow
Type III	Green

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.

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^{3,4}, but then you don't get a completely black background.

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, facilitates 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.

REFERENCES

Although this method is technically very easy, it is important for all involved using the stained slides, to have a basic understanding Picrosirius Red staining. Even without a polarizing microscope, Picrosirius Red shows things like reticular fibers and the basal lamina of cerebral capillaries, that 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. It is not advised that a Picrosirius Red stain be used without reading at least one of the first two items listed below.

1. Puchtler H, Waldrop FS, Valentine LS. *Polarization microscopic studies of connective tissue stained with picrosirius red* FBA. Beitr Path 1973; 150, 174-187
2. 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
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. Anique Ducharme, Stefan Frantz, Masanori Aikawa, Elena Rabkin, Merry Lindsey, Luis E. Rohde, Frederick J. Schoen, Ralph A. Kelly, Zena Werb, Peter Libby and Richard T. Lee. *Targeted deletion of matrix metalloproteinase-9 attenuates left ventricular enlargement and collagen accumulation after experimental myocardial infarction*. J Clin Invest. 2000;106(1):55-62. doi:10.1172/JCI8768¹.
6. Kiernan. J.A., (1999) *Histological and Histochemical Methods: Theory and Practice*, Ed. 3 Butterworth Heinemann, Oxford, UK.

ORDERING INFORMATION

Cat. #	Description	Size
24901-250	Picrosirius Red Stain Kit	250 ml
24901-500	Picrosirius Red Stain Kit	500 ml

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