BIOTnA Biotech

BIOSCIENCE FOR THE LAB.

Product Name: Picrosirius Red Stain Kit

Catalog No: TASS08

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 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 iron Hematoxylin for 8 minutes (optional) if Weigert's ironhematoxylin 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 light3,4, 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, 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.

Storage and Stability:

Store at 25-28℃. The user must validate any other storage conditions. When properly stored, the reagent is stable to the date indicated on the label. Do not use the reagent beyond the expiration date. If unexpected results are observed which cannot be explained by variations in laboratory procedures and a problem with the reagent is suspected, contact Technical: info@biotna.net