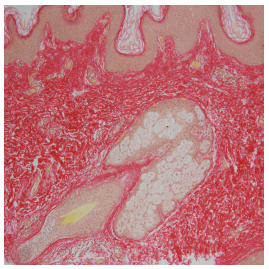
Aug 20, 2025

# Picrosirius red staining for histology



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**Protocol status:** Working
**We use this protocol and it's working**

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**Keywords:** histology, histochemistry, collagen, microscopy, pathology, histopathology, polarised, psr dye stains collagen fibre, histology picrosirius red, collagen in tissue section, iii collagen fibre, using polarised light microscopy, collagen, polarised light microscopy, histochemical technique for the identification, tissue component, picrosirius red, tissue section, fibrosi, tissue remodelling, histochemical technique, immunohistochemistry

## Abstract

Picrosirius red (PSR, aka Sirius red) staining is a histochemical technique for the identification of collagen and its specificity makes it suitable for robust quantitative assessments of collagen in tissue sections. Such digital image analysis is particularly valuable for the evaluation of fibrosis and tissue remodelling.

The PSR dye stains collagen fibres red, while also making them birefringent. There is some non-specific red staining of tissue components, which may include nuclei, keratohyalin granules and mucus, however these are not rendered birefringent so do not impact quantification using polarised light microscopy. The non-specific nuclear staining can be prevented by completing the optional heteropolyacid pre-treatment step in this protocol.

Some authors use PSR to distinguish between type I and type III collagen fibres, but recent analysis has shown that this is not always reliable, with immunohistochemistry providing a more definitive distinction.

## Protocol materials

- Phosphotungstic acid hydrate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**P4006
- Ethanol **POCD Scientific** **Catalog #**ETHABS20M
- Xylene **POCD Scientific** **Catalog #**XYL5P
- DPX Mountant for histology **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**06522
- Picric acid **May & Baker LTD**
- Sirius red F3BA **BDH Chemicals Ltd Poole England** **Catalog #**34149
- Iron (III) chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**F2877
- Hydrochloric Acid **Banksia Scientific Company (AJAX FineChem)** **Catalog #**AJA256
- Haematoxylin **Fronine Laboratory Supplies** **Catalog #**JL555S
- Ethanol **Hurst Scientific** **Catalog #**EIMS95-5L
- Ethanol **POCD Scientific** **Catalog #**ETHABS70WV5
- Glacial acetic acid **Ajax Finechem (ThermoFisher Scientific)** **Catalog #**AJA1-500ML

## Deparaffinisation and rehydration

32m

1 **Dewax and rehydrate tissue sections** 32m

## Heteropolyacid pre-treatment (optional)

3m 30s

2 Incubate in 0.2% phosphotungstic acid (PTA) solution 2m

PTA solution: 0.2 g PTA in 100 ml distilled water

Phosphotungstic acid hydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**P4006

Note

This PTA pre-treatment prevents non-specific nuclear staining in PSR, thereby enhancing the distinction between collagen and cellular staining. Elimination of the non-specific staining is particularly beneficial if performing automated digital image analysis, improving rigour of the method.

Citation

Mukade Y, Kobayashi S, Nishijima Y, Kimura K, Watanabe A, Ikota H, Shirabe K, Yokoo H, Saio M
(1970)
. Phosphotungstic Acid-treated Picrosirius Red Staining Improves Whole-slide Quantitative Analysis of Collagen in Histological Specimens.

https://doi.org/10.1369/00221554221141140 LINK

3 Rinse with distilled water 30s

4 Rinse with distilled water 30s

5 Rinse with distilled water 30s

## Nuclear staining (optional) 20m

6 Stain in Weigert's Haematoxylin (combine solution A & B immediately before use) 10m

Solution A: 1 g Haematoxylin in 100 ml of 95% Ethanol
Solution B: 1.6 g Ferric chloride hexahydrate (Iron III chloride; FeCl3.6H2O) + 95 ml distilled water + 1 ml concentrated HCl

- Haematoxylin **Fronine Laboratory Supplies Catalog #**JL555S
- Ethanol **Hurst Scientific Catalog #**EIMS95-5L
- Iron (III) chloride hexahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**F2877
- Hydrochloric Acid **Banksia Scientific Company (AJAX FineChem) Catalog #**AJA256

7 Wash with running tap water to remove excess stain 5m

8 Differentiate with acid alcohol (5-10 dips) to clear the slides

[1% Hydrochloric Acid (HCl) in 70% Ethanol]

- Hydrochloric Acid **Banksia Scientific Company (AJAX FineChem) Catalog #**AJA256
- Ethanol **POCD Scientific Catalog #**ETHABS70WV5

9 Wash with running tap water 5m

Note

It is important to ensure that the slide is completely cleared of excess Weigert's Haematoxylin at this point. If there is carryover into the PSR solution, it becomes contaminated with the ferric chloride, resulting in non-specific red background staining.

## Collagen staining

10 Stain with picrosirius red solution

1h

Solution: 0.1 g Sirius Red F3BA + 100 ml saturated aqueous picric acid

[M] 1.3 Mass Percent in distilled water

Sirius red F3BA **BDH Chemicals Ltd Poole England Catalog #**34149 CI: 35780

Picric acid **May & Baker LTD**

Sirius red is also known as "Direct Red 80"

Solution can be re-used many times. Lasts 20 years.

**Safety information**

Picric acid is an unstable explosive when dry, where it becomes highly sensitive to shock, heat and friction. Ensure it remains hydrated at all times.
Picric acid reacts with metals to form unstable picrate salts which are more explosive. Do not use metal containers, lids or racks.
Picric acid is toxic if swallowed, inhaled or absorbed through the skin.
Consult the MSDS and follow all recommended safety precautions.

## Differentiation of PSR

30s

11 Differentiate the PSR staining with 2 changes of a 0.5% acetic acid solution, 5-10 dips each.

30s

The timing of this differentiation (or number of dips) should be optimised for each specific tissue. Use a microscope to check that collagen is stained red and cells are stained yellow.

Solution: 0.5 ml glacial acetic acid + 99.5 ml distilled water

Glacial acetic acid **Ajax Finechem (ThermoFisher Scientific) Catalog #**AJA1-500ML

12 Drain acetic acid solution from the slides as much as possible

## Dehydration, clearing and mounting

11m 30s

13 100% ethanol

30s

Note: If you want to maintain the yellow cytoplasmic staining, ensure the dehydration is quick.
10 dips per ethanol step is usually sufficient.

Ethanol **POCD Scientific Catalog** #ETHABS20M

14 100% ethanol 30s

Ethanol **POCD Scientific Catalog** #ETHABS20M

15 100% ethanol 30s

Note: ensure whole slide and slide rack is fully immersed in ethanol to remove all traces of water, preventing water contamination of the xylene

Ethanol **POCD Scientific Catalog** #ETHABS20M

16 100% Xylene 5m

Xylene **POCD Scientific Catalog** #XYL5P

17 100% Xylene 5m

Xylene **POCD Scientific Catalog** #XYL5P

18 Coverslip with a resinous mounting medium, such as DPX

DPX Mountant for histology **Merck MilliporeSigma (Sigma-Aldrich) Catalog** #06522

## Results

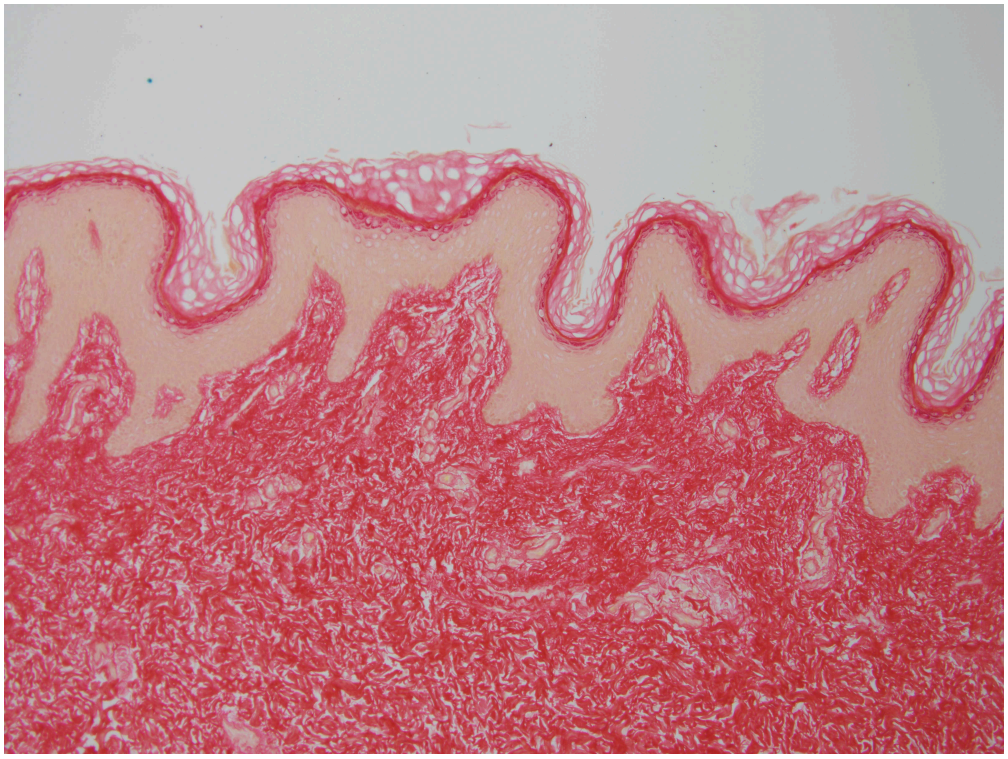
19

## Expected result

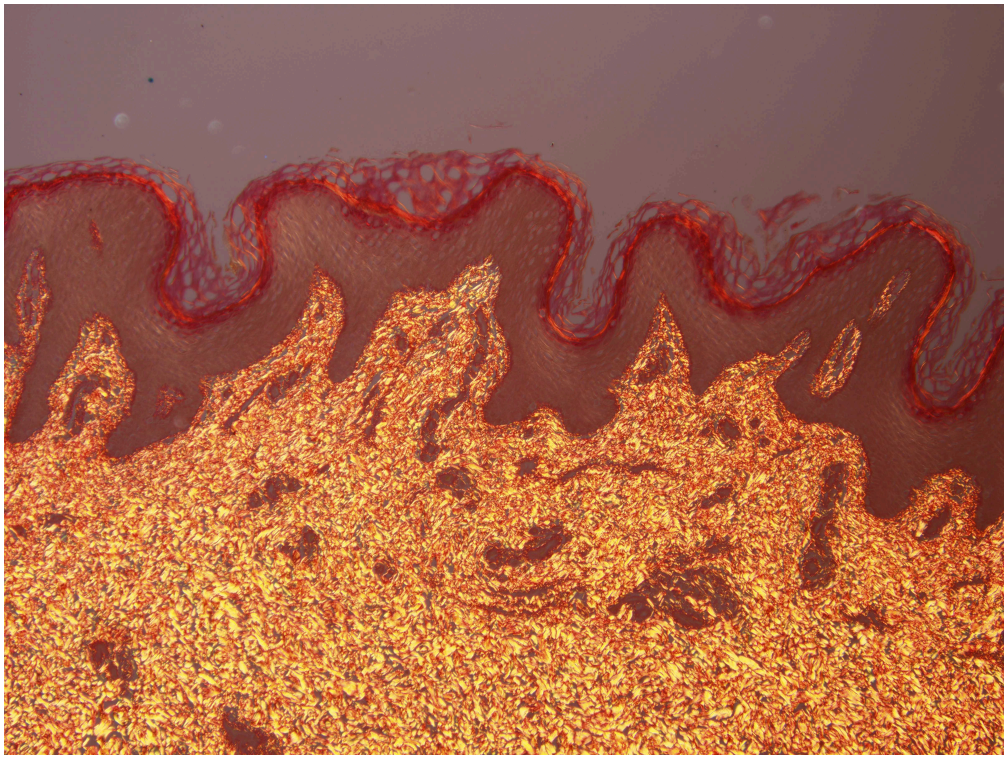
Collagen: Red
Bone: Red
Muscle: Yellow
Epithelium: Yellow
Erythrocytes: Yellow
Nuclei: Brown - Black

Note - collagen and bone stained specifically with Sirius Red are birefringent, enabling identification and quantification under polarised light microscopy.

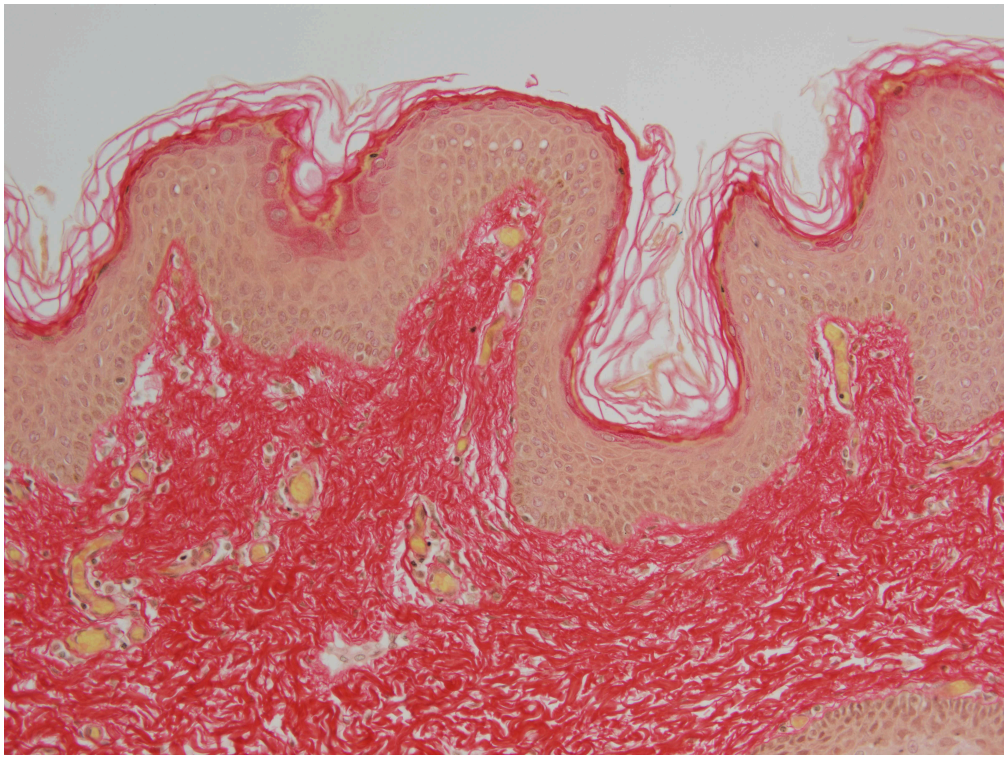
In the below images, the optional heteropolyacid pre-treatment steps were not used.



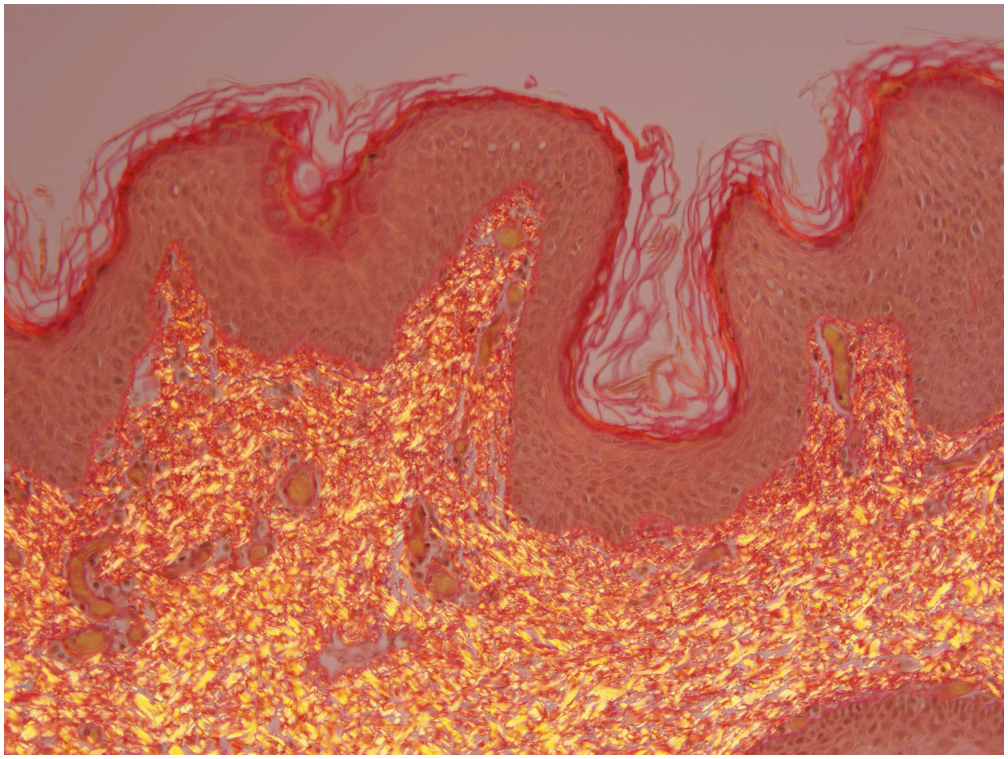
Skin tissue stained with PSR, without nuclear counterstain. Imaged with regular brightfield microscopy.



Skin tissue stained with PSR, without haematoxylin nuclear counterstain. Imaged with polarised light microscopy. Note that while keratin stains red with PSR, it does not exhibit birefringence as it is not collagenous.



Skin tissue stained with PSR, with Weigert's haematoxylin nuclear counterstain. Imaged with regular brightfield microscopy.



Skin tissue stained with PSR, with Weigert's haematoxylin nuclear counterstain. Imaged with polarised light microscopy. Note that while keratin stains red with PSR, it does not exhibit birefringence as it is not collagenous.

## Protocol references

López De Padilla, C. M., Coenen, M. J., Tovar, A., De la Vega, R. E., Evans, C. H., & Müller, S. A. (2021). Picrosirius Red Staining: Revisiting Its Application to the Qualitative and Quantitative Assessment of Collagen Type I and Type III in Tendon. *The journal of histochemistry and cytochemistry : official journal of the Histochemistry Society*, *69*(10), 633–643. https://doi.org/10.1369/00221554211046777

## Citations

Step 2

Mukade Y, Kobayashi S, Nishijima Y, Kimura K, Watanabe A, Ikota H, Shirabe K, Yokoo H, Saio M. Phosphotungstic Acid-treated Picrosirius Red Staining Improves Whole-slide Quantitative Analysis of Collagen in Histological Specimens.
**https://doi.org/10.1369/00221554221141140**