# Overview

This document explains how to prepare, cut, mount and stain cardiac tissue for picrosirius red staining. Use this stain to identify collagen in tissue. The protocol walks through how to do this using human heart tissue from the cardiac bank.

# Additional resources

* [Picrosirius Staining Revisited](https://teams.microsoft.com/l/file/49CBA2DB-89E0-4032-8531-2E85FCA1D801?tenantId=2b30530b-69b6-4457-b818-481cb53d42ae&fileType=pdf&objectUrl=https%3A%2F%2Fluky.sharepoint.com%2Fsites%2FCampbellLab%2FShared%20Documents%2FProtocols%2FHistology%2FPicrosirius%20Red%20Staining%20Revisited.pdf&baseUrl=https%3A%2F%2Fluky.sharepoint.com%2Fsites%2FCampbellLab&serviceName=teams&threadId=19:77468b43cd88423598343156f29c1b0c@thread.skype&groupId=4e4675c3-ea35-4036-9b4c-2ace772cc6af)
* [Quantitative Assessment of Myocardial Collagen with Picrosirius](https://teams.microsoft.com/l/file/74BE1841-D63C-488D-B862-257128C81EA0?tenantId=2b30530b-69b6-4457-b818-481cb53d42ae&fileType=pdf&objectUrl=https%3A%2F%2Fluky.sharepoint.com%2Fsites%2FCampbellLab%2FShared%20Documents%2FProtocols%2FHistology%2FQuantitative%20assessment%20of%20myocardial%20collagen%20with%20picrosirius.pdf&baseUrl=https%3A%2F%2Fluky.sharepoint.com%2Fsites%2FCampbellLab&serviceName=teams&threadId=19:77468b43cd88423598343156f29c1b0c@thread.skype&groupId=4e4675c3-ea35-4036-9b4c-2ace772cc6af)
* [Picrosirius Red Staining- A Useful Tool to Appraise Collagen Networks in Normal and Pathological Tissues](https://teams.microsoft.com/l/file/EE0A4165-002F-4A18-ADD0-B3BCF1CB91AA?tenantId=2b30530b-69b6-4457-b818-481cb53d42ae&fileType=pdf&objectUrl=https%3A%2F%2Fluky.sharepoint.com%2Fsites%2FCampbellLab%2FShared%20Documents%2FProtocols%2FHistology%2FPicrosirius%20Red%20Staining-%20A%20Useful%20Tool%20to%20Appraise%20Collagen%20Networks%20in%20Normal%20and%20Pathological%20Tissues.pdf&baseUrl=https%3A%2F%2Fluky.sharepoint.com%2Fsites%2FCampbellLab&serviceName=teams&threadId=19:77468b43cd88423598343156f29c1b0c@thread.skype&groupId=4e4675c3-ea35-4036-9b4c-2ace772cc6af)

# Main content

**Part 1: Staining**

**Note: Do these steps in the fume hood.**

* While waiting for the slides to air dry, ensure that the water bath is set to 56 degrees Celsius.
* Place coplin jar filled with Bouin’s solution into the water bath at 56C 30 mins prior to start of staining to allow Bouin’s to be at desired temperature.
  + Beware of the fumes - Bouin's contains picric acid and formaldehyde, which is very toxic!
* Place slides into coplin jar in the water bath and incubate for 1 hour.
* Wash the slides two times with DI water
  + You can use two beakers one for each wash.
* The sirius red is made by weighing 0.5g of Direct Red 80 into 500 mL of saturated picric acid solutions (1.3%)
  + Note: This solution is good for approximately 3 years and can be used multiple times.
* Place the slides into coplin jars that contain the sirius red stain. Let these incubate on a rocker for 2 hours.
  + Ensure that the stain is high enough to cover all of the tissue.
* Wash the slides 2 times in 0.5% acetic acid for 10s
  + 500 uL of acetic acid into 100 mL of Water
  + Note: Frequently replace the acetic acid solution used for washes once the solution becomes red with residual picrosirius red stain (be liberal with the amount of wash changes for best results).
* Dehydrate the slide with 2x 20s dips in 95% ethanol followed by 2x 20s dips in the 100% ethanol.
  + Note: Like the acetic acid washes, replace solutions if they become too red with stain
* Place the slides in xylene for ~2 mins
* Let slides dry completely before proceeding with coverslipping

**Part 2: Coverslipping**

* Dehydrate the slide again with a 20s wash in 100% ethanol
* Clear the slide again with a 20s wash in Xylenes
  + NOTE: do not let Xylene dry before applying Perimount. Xylene helps to liquify the Perimount and allow it to spread across the tissue underneath the coverslip.
* Mount the slides with xylene based mounting media and cover slip
  + Apply a small amount of **Perimount** to the bottom of the slide, grab a glass coverslip, position edge of coverslip at the bottom of the slide and place top of coverslip at top edge of slide thus pushing mounting agent up across the full section(s).
  + Gently press out any and all air bubbles with pipette tip while the mounting media is liquid.
* Allow to dry for approximately 1 day
* Clean the slides before use with ethanol or xylene if there are glue smudges

**Protocol Summary Table**

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| **Picrosirius Red Staining Protocol**  Section thickness = 10μm | |
| *Turn on water bath to 56°C and place coplin jar containing Bouin’s solution into bath to heat up before starting anything else* | |
| **Bouin’s Solution @ 56°C** | 1 hour |
| Wash in 2x changes of **fresh diH2O** | ~30 secs/each |
| **Picrosirius Red Stain** (place on rocker) | 2 hours |
| Wash in 2x changes of **0.5% Acetic Acid** | ~10 secs/each |
| *Graded Dehydration Steps* | |
| 95% Ethanol | ~20 secs |
| 95% Ethanol | ~20 secs |
| 100% Ethanol | ~20 secs |
| 100% Ethanol | ~20 secs |
| Air dry completely with air hose before clearing in Xylene | |
| *Clearing & Mounting Steps* | |
| **Xylene** | 10-15 mins |
| Air dry completely with air hose. | |
| Place in **Xylene** again for a few seconds before applying mounting medium. | |
| Add **DPX** (mounting medium) while xylene is freshly wet and apply cover glass. | |