# 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: Cryostat**

* Collect the following:
  + Cryostat molds
    - You should use a sharpie to write the hashcode on the edge of the molds BEFORE you attempt to make the molds
  + Razor blade
  + Forceps
  + Weigh boat for heart tissue
  + OCT compound
  + Styrofoam container with dry ice
  + Liquid nitrogen cooled Isopentane
* Obtain the appropriate sample(s) from the cardiac biobank
  + Place the samples on the dry ice
* Remove the heart sample from the cryogenic vial and place it on the weight dish.
  + Have the corresponding cryomold ready, so that you do not mix up which sample should go in which mold.
* Carefully use the forceps and razor blade to cut a piece of tissue that will fit inside the cryomold
  + Preferably a piece that will not touch any of the edges of the mold.
  + Smaller pieces of tissue freeze better because they freezer quicker and more evenly which minimized freezing artifacts
* Place one small dot of OCT compound in the center of the mold, then place the tissue in the mold and fill the rest of the mold with the OCT compound.
* Place the mold into the liquid nitrogen cooled isopentane for 15 secs
  + Be careful to ensure that the mold lays evenly so that the OCT compound does not fall out.
* Put unused tissue back into the vial and place the vial on the dry ice.
* Place molds into -80°C freezer for 30 mins.
* Move molds to cryostat at -20°C for 30 mins prior to cutting.
* Once tissue has equilibrated for 30 mins in cryostat, take one of the chucks, and apply a nickel size dot on the chuck and place the prepared sample on it, and allow that to freeze completely.
  + Notes:
    - The dot will freeze quickly, so you need to have the sample already taken out of the mold, and ready to place on the platform
    - Ideally position the sample block level on the platform such that the base of the sample is parallel to the platform surface.
    - Keep the mold so that you can place the unused sample back in
    - It is not wise to have more than one sample outside of the molds at a time, because they will become easy to switch up.
* While you are waiting for the sample to freeze to platform note if you need a new blade. You do not need a new blade every time you use the cryostat but if you are getting tears in the tissue, it may be due to an old, dented blade.
  + There is a lever to the left or right of the blade that can be moved to loosen the blade.
  + Use forceps to remove the old blade and add in the new blade.
  + Retighten the blade using the lever described previously
* Once completely frozen, insert the platform and adjust as necessary
* To begin, you will need to move the block toward the blade.
  + There is a move forward button that you can use so that you do not need to crank it all the way to the blade, but be careful, because if you go too far you can cut right into the middle of the sample and ruin it, or it will come off the block all together, and might be lost.
* Once you begin to see complete sections, you can attempt to put them on the slide
  + Notes: You will need to put down the glass roll protector to see if you are getting a complete section
  + You should only have one slice on the platform when trying to put the slice on the slide.
  + You want to hover the slide without actually touching it to the platform
    - The sample section should rise up to adhere to the slide. It may be helpful to flip the section so that the section buckles upwards or at least is in no way stuck to the cryostat.
  + DO NOT keep the slides in the cryostat, because it they get too cold the section will not adhere to the slide.
* Repeat for all of the required sections then allow to air dry for 1 hour.

**Part 2: 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 until equilibrated (~2-5 mins)
* 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