# 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)

# Need more help?

Check the resources, and then see Ken

# 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
* 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
* 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 on the dry ice
  + 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.
* Allow the molds to completely freeze.
  + They are done when they turn completely white.
  + While you are waiting for the molds to freeze completely you should:
    - Check to make sure that the settings on the cryostat are set appropriately
    - Label the appropriate number of microscope slides with the hashcode(s)
* Once at the cryostat, take one of the platforms, and apply a nickel size dot on the platform 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, you should put in a new blade for the cryotstat, so that you do not contaminate your sections.
  + 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 the slides in holder box that will fit in the water bath.
* Use a disposable pipette to drop the Bouin's fixative onto the slides in the box. Make sure that you use plenty to completely cover the tissue.
  + If the fixative dries on the tissue, it will not stain properly.
  + Beware of the fumes - Bouin's contains picric acid and formaldehyde, which is very toxic!
* Let the fixed slides sit in the water bath 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 couplin 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
  + 500 uL of acetic acid into 100 mL of Water
  + 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 a couple of dips in 95% ethanol followed by a couple of dips in the 100% ethanol.
  + 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