

## Hematoxylin-Phyloxine-Saffron (HPS) stain

Deparaffinize slides and bring to water

1. Place slides in Bouin's solution for 1 minute
  - a. Heat Bouin's solution to 60C (microwave for 30 seconds and place in 60C oven)
2. Wash Bouin's solution off with distilled water
  - a. Bouin's solution will take long to come off tissue – several minutes. Make sure the yellow stain is off the slides.
3. Place slides in hematoxylin for 2:30
4. Wash off hematoxylin for 1 minute
5. Place slides in clearview for 30 seconds
6. Wash off clearview for 30 seconds
7. Place slides in bluing solution
8. Wash off bluing solution for 30 seconds
9. Place slides in Solution A for 7 minutes
  - a. 100mL distilled water and 2g of Phyloxine B
  - b. Filter solution
  - c. Add 3 drops of formalin for preserving solution
10. Wash off Solution A for 30 seconds
11. Dehydrate slides
12. Place slides in Solution B for 7 minutes
  - a. 200mL absolute ethanol and 6g saffron
  - b. Seal tightly and place in 60C over for 1-2 weeks
13. Wash Solution B off with 2 changes of 100% ethanol
14. Bring slides to xylene
15. Coverslip slides and dry

Results:

Nuclei – blue

Cytoplasm – red shads

Muscle – pink

Elastic fibers – bright red

Collagen – yellow

Notes:

1. If the saffron solution is too strong it may be diluted with absolute ethanol. Store and use at room temperature. It has a limited life of about a month and is best when freshly made. It is important that there be no water in the saffron solution and that sections be thoroughly dehydrated before it is applied. If contaminated with moisture the solution must be discarded.