**Medeiros Lab Paraffin Embedding Protocol**

**Weekend Before Day 1**

1. Retrieve samples
2. Cut samples to size and put in SafeFix for the weekend

**Day 1-Dehydration**

1. Dehydrate tissue using the following washes using 190 proof EtOH:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **%EtOH** | **Measurements using 95% EtOH** | **Time** | **Temperature** | **Mode** |
| 30% | 63ml EtOH/ 137 ml diH2O | 1 hour | RT | Rocking |
| 50% | 105ml EtOH/ 95ml diH2O | 1 hour | RT | Rocking |
| 50% | 105ml EtOH/ 95ml diH2O | 1 hour | RT | Rocking |
| 60% | 126ml EtOH/ 74ml diH2O | 1 hour | RT | Rocking |
| 70% | 147ml EtOH/ 53ml di H2O | Overnight | RT | Rocking |

**Day 2-Fixation**

4-5 Using 190 proof EtOH:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **%EtOH** | **Measurements using 95% EtOH** | **Time** | **Temperature** | **Mode** |
| 85% | 179mL EtOH/ 21mL diH2O | 1 hour | RT | Rocking |
| 95% | 200mL EtOH | 1 hour | RT | Rocking |

6. Replace 95% Ethanol with 100% Ethanol (using 200 proof) and incubate for 1 hour, rocking.

7. Remove as much of the 100% Ethanol as possible and replace it with fresh 100% Ethanol. Incubate for 30min at RT.

8. Repeat Step 7

9. Remove the Ethanol and do the following washes:

|  |  |  |  |
| --- | --- | --- | --- |
| **SafeClear II:Ethanol** | **Time** | **Temperature** | **Mode** |
| 25%:75% | 1 hour | RT | Rocking |
| 50%:50% | 1 hour | RT | Rocking |
| 75%:25% | 1hour | RT | Rocking |

10**.** Replace the 75%:25% solution with 100% SafeClear II and incubate for 1 hour at RT.

11. Repeat Step 10

12. Repeat Step 10 again. After incubation half-fill the jar with SafeClear II.

13**.** Add about 40 Paraplast chips to the jar and incubate overnight at room temperature.

14. Prepare molten Paraplast for Days 3-4 by filling 4 jars with Paraplast chips and placing it at 60°C. After they have melted some, top off with more Paraplast chips.

*After the overnight incubation, the Paraplast in SafeClearII will be in solution only partially.*

**Day 3-Fixation with Paraplast**

15.Prepare hot water bath using the fryer in the fume hood. Put foil down into the water to avert steam from the sample jar. Put a beaker with water and a thermometer in to monitor temperature. Temperature needs to hang around 39-42ºC. Turn the on dial just until the light comes on.

16. Place sample jar with SafeClear II/Paraplast chip solution into hot water bath. Let Paraplast chips completely melt.

17.Add about 40 more chips, and incubate at 42ºC until dissolved (~10-15 min). Swirl occasionally.

18. Repeat four to five times.

19.Remove the SafeClearII/Paraplast solution and replace with molten Paraplast. Swirl to mix. Incubate for at least 4 hours at 57ºC-62ºC.

20. Replace molten Paraplast with fresh and incubate at 57ºC-62ºC overnight.

**Day 4-Paraplast and Prep**

21. Replace molten Paraplast with fresh and incubate at 57ºC-62ºC for at least 4 hours.

22. Prepare for Day 5:

-Place bead tray in 60ºC oven for Day 5.

-Place 1 canning jar with Paraplast chips in 60ºC oven for Day 5.

-Turn on and stabilize the vacuum oven.

-Turn on the Shandon Histocentre to melt the Paraplast; check and top off with more if needed

23. Replace molten Paraplast with fresh and incubate at 57ºC-62ºC overnight.

**Day 5-Embedding**

24. Transfer the cassettes to a fresh jar of molten Paraplast

25. Put jar into bead tray for transport to vacuum oven. Loosen lid.

26. Put jar into oven (leave in bead tray). Close door and latch completely.

27. Tighten vent. Turn vacuum on. Wait until pressure reaches 15Hg, and then close it off. Leave jar in oven for two hours.

28. Turn on warming tissue tank while you wait. Label embedding rings and set up in the room with the paraffin dispenser.

29. Turn on cold plate about a half-hour before needed.

30. Remove bead tray and jar from oven and transfer cassettes to warming tissue tank.

31. Put warmed mold under dispenser. Dispense a little paraffin.

32. Remove tissue sample from cassette and hold in position in the mold. Move to cold plate.

33. Hold on cold plate until sample can stand up on its own. Put plastic ring on and press down. Move to hot plate and let melt a little for a seamless block.

34. Use dispenser to fill to the top of the ring. Move to cold plate to harden.