Heart Removal

1 Remove heart from rat immediately following sacrifice
Rinse heart in 1X PBS for 00:00:30 to flush blood from the chambers
Embedding Heart

1. Place heart (still beating) in solution of 1X PBS with 25% Optimal Cutting Temperature Compound (OCT) for 00:00:01 with gentle agitation.

2. Place heart in solution of 1X PBS with 50% OCT for 00:00:45.

The heart should still be beating at this point, albeit weaker. This will allow for OCT infiltration into the chambers in order to mitigate any structural collapse while sectioning.

3. Place heart in the embedding mold containing a layer of frozen 100% OCT at bottom.

This layer of OCT is to help prevent the heart from sinking to the bottom of the mold exposing the tissue. All areas of the tissue sample should be covered with OCT to allow for optimal sectioning capability.

4. Flash freeze the embedding mold containing heart in 100% OCT by suspending mold in either liquid nitrogen or methanol in dry ice.

Be careful to not allow any liquid nitrogen or methanol to splash into the embedding mold. This will make the OCT become a gooey texture and very difficult to section.

5. Store heart in -80 °C freezer wrapped in aluminum foil and plastic wrap until the sectioning process is started.

Allow heart to sit in -80 °C freezer for at least one day before beginning to section.

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