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OSU TriState SenNet Processing and Storing of a Normal Donor Heart

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Cellular Senescence Net…

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We use this protocol and it's working

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Disclaimer

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Abstract

This protocol describes the processing and storing of normal donor heart by the Comprehensive Transplant Center (CTC) Human Tissue Biorepository and at the TriState SenNet Biospecimen Core of The Ohio State University that functions as a part of the Cellular Senescence Network Program (SenNet). This protocol is based on the CTC Human Tissue Biorepository's standard operating procedure established for tissue collection for explant and donor samples. We follow routine laboratory specimen processing guidelines to ensure the highest biospecimen quality.

Materials

·Nitrile gloves
·Disposable gown
·Biosafety cabinet (BSC)
·Underpads (blue absorbent pads)
·Large and small biohazard bags
·Small biohazard bag holder, used to hold the bag in BSC for ease and efficiency
·Nalgene biohazard bin lined with red medium biohazard bag
·Sterilized surgical kit containing 2 surgical scissors, 2 forceps, and 2 hemostats
·Cryovial rack
·2 mL cryovials
·4% paraformaldehyde (PFA)
·70% ethanol
·Tissue cassettes
·Optimal cutting temperature compound (OCT)
·OCT mold
·30% sucrose in PBS
·50ml conical tubes

Objective

1 To preserve heart tissue for further downstream cellular, protein, RNA, or DNA analyses.

Preparation

2 In the biosafety cabinet (BSC), place three underpads and all needed equipment, including biohazard receptacles, surgical kits, and specimen holders. Before beginning processing samples, check that all appropriate personal protective equipment is donned and that BSC airflow is on.

3 A donor heart comes triple-bagged in HTK solution or saline from our local organ procurement organization (Lifeline of Ohio). Place the container holding the specimen inside the BSC, remove the heart from containers, and place the heart on the underpad.

4 For normal donor samples, the heart tissue is reddish-purple in color with white chordae located within. Healthy tissue feels firm and robust. When processing normal donor tissue, look for the healthiest region to sample. The donor's CT or chest X-ray can provide information on selecting the healthiest regions. Select the sites from which samples will be collected and annotate them in the notebook.

5 From the heart, remove a 2 cm^3 piece of tissue from the septum, left and right ventricles, and the left and right atrium.

Freezing and storing

6 Cut 7 small pieces of tissue (4mm^3) from each region (septum, right and left ventricles, right and left atrium), and place them onto the internal sides of a labeled cryovial using forceps. Collect cryovials of heart tissue from each region.

7 Lower the cryovials into liquid nitrogen and leave for 5 minutes.

8 After freezing, remove the cryovials from liquid nitrogen and promptly store them in a -80°C freezer.

Storing for Formalin-fixed Paraffin-embedded (FFPE) and OCT sample processing

9 **For FFPE sample storage:**

9.1 Add mL 4% paraformaldehyde (PFA) into a labeled 50ml conical tube, and place two 25 cm^3 pieces of tissue from each region of the heart into separate tubes.

9.2 Keep samples away from light at 4°C for 24 hours. After 24 hours, remove the PFA and replace with 70% ethanol for another 24 hours.

9.3 Remove the tissue from PFA and cut the tissue into a < 2mm thick piece that is small enough to fit into the tissue cassette. Tissue should be 1.5 cm x 1.25 cm.

9.4 Enclose the tissue into the cassette, and then fully submerge the cassette in a 50 mL conical with 70% ethanol.

9.5 Embedding and histology are performed using standard methods.

10 **For OCT embedded samples:**

10.1 Cut the tissue into 1 cm x 1 cm pieces.

10.2 Transfer the piece of tissue into 4% PFA. Keep samples away from light at 4°C for 24 hours.

10.3 After hours, transfer to a 30% sucrose and PBS solution at 4°C for 24 hours, and then embed in a mold with OCT.

10.4 Store samples in a -80°C freezer.