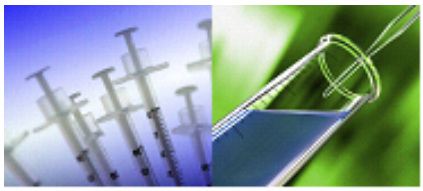

nPOD
Network for Pancreatic Organ Donors with Diabetes

RNA EXTRACTION

1 PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to outline procedures for extracting high quality RNA from nPOD samples.

2 SCOPE

This SOP will be applied to the extraction of RNA from fresh or snap frozen tissue and OCT-embedded frozen blocks.

3 RESPONSIBILITIES

3.1 Managers and supervisors are responsible for making sure that technicians are properly trained and equipment and facility are maintained in good working order.
3.2 Laboratory personnel are responsible for reading and understanding this SOP and related documents and to perform these tasks in accordance with the SOPs. They are responsible for following clinical laboratory and tissue banking best practices.

4 EQUIPMENT and MATERIALS

The materials, equipment and forms listed in the following list are recommendations only and alternative products as suitable may be substituted for the site specific task or procedure.

Qiagen RNeasy Plus Mini Kit (Qiagen, Cat. No. 74134)
2-Mercaptoethanol (β-ME)
70% Ethanol (molecular biology grade, RNA free)
Microcentrifuge, VWR Pellet Mixer
RNase-free Pestle & Microtube Combo 1.5ml
Microcentrifuge, Spectrophotometer
Agilent 2100 bioanalyzer & Agilent RNA 6000 Nano Kit (Cat. No. 5067-1511)

5 SAFETY

5.1 Use universal safety precautions when handling human samples and personal protective equipment (e.g., face mask with shield, gloves, lab coat or apron).

6 PROCEDURE

6.1 RNA Extraction from Tissue and Snap-frozen vials.
- 6.1.1 Place tissue in 1.5ml RNase-free microtube.
  - 6.1.1.1 Use about 20mg of pancreas tissue.
  - 6.1.1.2 Mince further with sterile scalpel to aid tissue disruption.
- 6.1.2 Add 500ul of Buffer RLT Plus.
  - 6.1.2.1 10µl of β-ME per 1ml of Buffer RLT Plus must be added before Buffer is used.

6.1.3 Immediately disrupt and homogenize the tissue until it is uniformly homogenous, approximately 40 seconds, using the Pellet Mixer and RNase-free pestle.
6.1.4 Add another 100µL of Buffer RLT Plus.
6.1.5 Centrifuge the lysate for 10 minutes at maximum speed.
6.1.6 Remove the supernatant by pipetting, and transfer to a gDNA Eliminator spin column placed in a 2 ml collection tube.
6.1.7 Centrifuge for 30s at ≥8000g.
6.1.8 Discard the column and save the flow-through.
6.1.9 Add 600µl of 70% ethanol to the flow-through and mix well by pipetting.
6.1.10 Transfer 600µL of the sample, including any precipitate, to an RNeasy spin column placed in a 2ml collection tube, close the lid gently.
6.1.11 Centrifuge for 15s at ≥8000 x g.
6.1.12 Repeat Steps 6.1.10 and 6.1.11 for the successive aliquots in the same RNeasy spin column, discarding the flow-through after each centrifugation.
6.1.13 Add 700µL Buffer RW1 to the RNeasy spin column, close lid gently.
6.1.14 Centrifuge for 15s at ≥8000 x g. Discard flow-through.
6.1.15 Add 500µl Buffer RPE to the RNeasy spin column, close lid gently.
6.1.16 Centrifuge for 15s at ≥8000 x g. Discard flow-through.
6.1.17 Add 500µl Buffer RPE to the RNeasy spin column, close lid gently.
6.1.18 Centrifuge for 2 min at ≥8000 x g. Discard flow-through and collection tube.
6.1.19 Place the RNeasy spin column in a new 2 ml collection tube.
6.1.20 Centrifuge for 1 min at full speed. Discard flow-through and collection tube.
6.1.20.1 Do not allow column to contact flow through.
6.1.21 Place RNeasy spin column in a new 1.5 ml collection tube.
6.1.22 Add 30µl of RNase-free water directly to the spin column.
6.1.23 Centrifuge for 1 min at ≥8000 x g to elute the RNA.

6.2 RNA Extraction from OCT Blocks

6.2.1 Section tissue, approximately 50µm, under RNase-free conditions and place in RNA-free microfuge tube. Continue as for tissue described above.

6.3 Quantification and Quality Analysis

6.3.1 Determine RNA quantity using spectrophotometer by measuring optical density at A260nm. Determine the A260/A280 nm wavelength ratio, which should be ~2.0 for high quality RNA preparations.
6.3.2 Save all raw data. Import into the RNA Calculator Excel file to show A260/A280 ratio, stock RNA concentration (ng/µl), extraction volume (µl), and total RNA yield (µg).
6.3.3 Analyze extracted RNA quality using electrophoresis, Agilent 2100 Bioanalyzer and Agilent RNA Nano Kit. Following Agilent RNA 6000 Nano Kit Quick Start Guide for RNA microfluidic chip preparation.
6.3.4 Save all raw data. Import RIN values to the RNA Calculator Excel file.
6.3.5 Freeze RNA and store at -80°C.

7 REFERENCES

7.1 Qiagen RNeasy Mini kit protocols

8 REVISION HISTORY

Version	Date	Revision

Prepared by	Tiffany Heiple		
Approved by	Martha Campbell-Thompson		
	Name	Signature	Date