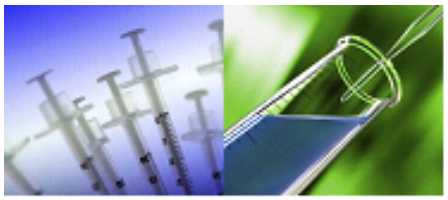

nPOD
Network for Pancreatic Organ Donors with Diabetes

STANDARD OPERATING PROCEDURE

RNA Extraction

OPPC-SOP-81

Prepared by:	Tiffany Heiple	**Original Effective Date:**	10/12/2012
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Network for Pancreatic Organ Donation with Diabetes (nPOD)
BMSB Room J586
P.O. BOX 100275
Gainesville, FL 32610

RNA EXTRACTION

POLICY: Use universal safety precautions when handling human samples and personal protective equipment (e.g., face mask with shield, gloves, lab coat or apron). Buffers RTL Plus or RW1 supplied by Qiagen contain a guanidine salt and are not compatible with bleach.
2-Mercaptoethanol (β-ME) is highly combustable, toxic if swallowed, and can be fatal through skin contact. Handle in accordance with good industrial hygiene and safety practice and keep container tightly closed in a dry, cool, well-ventilated place away from sources of ignition.

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

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

RESPOSIBILITIES: Managers and supervisors - are responsible for making sure that technicians are properly trained and equipment and facility are maintained in good working order.

Laboratory personnel - are responsible for reading and understanding this SOP and related documents and to perform these tasks in accordance with the SOPs.

EQUIPMENT & 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) (Fisher, Cat.No. O3446I-100)
- 70% Ethanol, Molecular Biology Grade
- RNase-free water, Molecular Biology Grade
- Cordless Pestle Motor (VWR, Cat. No. 47747-370)
- RNaseZap Decontamination Solution (Ambion, Cat. No. AM9780)
- RNase Away Surface Spray (Thermo, Cat. No. 21-402-178)
- DNase-, RNase-, pyrogen-free Pestle & Microtube 1.5ml (VWR, Cat. No. 47747-366)
- Disposable Scalpel, #11 (FisherSci, Cat. No. NC0134996)
- Microcentrifuge
- Microcentrifuge tube racks
- Spectrophotometer (BioTek Epoch)
- Take3 Micro-volume plate (BioTek)
- Agilent 2100 bioanalyzer
- Agilent RNA 6000 Nano Kit (Agilent, Cat. No. 5067-1511)
- Micropipettes and RNase-free filter pipette tips (10 µL, 200 µL, 1000 µL)
- Pipet-Aid™ (Drummond XP, Cat. No. 4000101)
- Serological pipettes (5mL, 10mL)

- Disposable laboratory coats
- Nitrile gloves
- Safety glasses

PROCEDURE:

1.0 RNA Extraction from Tissue and Snap-frozen vials

1.1 Place 15-20 mg of pancreas tissue in 1.5ml RNase-free microtube and mince with sterile scalpel.

1.2 Add 500µl of Buffer RLT Plus.

1.3 10µl of β-ME per 1ml of Buffer RLT Plus must be added before buffer is used.

1.4 Immediately disrupt the tissue until it is uniformly homogenous, approximately 40-60 seconds, using the cordless pestle motor and RNase-free pestle.

1.5 Add another 100µL of Buffer RLT Plus and mix thoroughly.

1.6 Centrifuge the lysate for 3-10 minutes at maximum speed (>20,000 x g). Perform all centrifugation steps at 20-25°C.

1.7 Remove the supernatant by pipetting and transfer to a gDNA Eliminator spin column placed in a 2 ml collection tube.

1.8 Centrifuge for 30s at ≥8000 x g. Discard the column and save the collection tube with flow-through.

1.9 Add 600µl of 70% ethanol to the flow-through and mix well by pipetting.

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 prior to all centrifugation steps.

1.11 Centrifuge for 15s at ≥8000 x g. Save the column and collection tube and discard the flow-through. Do not allow column to contact discarded flow-through in any subsequent steps.

1.12 Repeat Steps 1.10 and 1.11 for successive aliquots in the same RNeasy spin column.

1.13 Add 700µL Buffer RW1 to the RNeasy spin column.

1.14 Centrifuge for 15s at ≥8000 x g. Discard flow-through.

1.15 Add 500µl Buffer RPE to the RNeasy spin column.

1.16 Centrifuge for 15s at ≥8000 x g. Discard flow-through.

1.17 Add 500µl Buffer RPE to the RNeasy spin column.

1.18 Centrifuge for 2 min at ≥8000 x g. Discard flow-through and collection tube.

1.19 Place the RNeasy spin column in a new 2 ml collection tube and centrifuge for 1 min at maximum speed. Discard flow-through and collection tube.

1.20 Place RNeasy spin column in a new 1.5 ml collection tube. Add 30µl of RNase-free water directly to the spin column.

1.21 Centrifuge for 1 min at ≥8000 x g to elute the RNA.

1.22 Continue with step 3.0 Quantification and Quality Analysis.

2.0 RNA Extraction from OCT Blocks

2.1 Section tissue, approximately 50µm, under RNase-free conditions and place in RNA-free microfuge tube. Continue with step 1.1 as for tissue described above.

3.0 Quantification and Quality Analysis

3.1 Determine RNA quantity using spectrophotometer by measuring optical density at 260nm and 280nm. Determine the ratio of A260/A280, which should be ~2.0 for high quality RNA preparations.

3.1.1 Place 2µl of sample on microplate. Use 2µl RNase-free water for blank.

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).

3.3 Analyze extracted RNA quality using Agilent 2100 Bioanalyzer and Agilent RNA 6000 Nano Kit. Follow procedure as written in Nano Kit.

3.3.1 Heat denature samples and RNA ladder in heating block at 70°C for 2 minutes, then place directly on ice until analysis.

3.3.2 Let all RNA 6000 reagents equilibrate to room temperature for 30 minutes prior to use.

3.3.3 Before analysis, decontaminate Bioanalyzer electrodes by pipetting 350µl RNaseZap into the electrode cleaning chip. Immerse electrodes for 60 seconds. Follow with 350 µl RNase-free water for 10 seconds.

3.3.4 After analysis, decontaminate using 350 µl RNase-free water for 10 seconds.

3.4 Save all raw data files in OPPC RNA folder on G: drive and nPOD SharePoint. Import RIN values to nPOD Access Database -> RNA table.

3.5 Freeze aliquots and store at -80°C according to SOP Tissue Sample Archiving.

REFERENCES:

1.0 Related Documents and Procedure

1.1 Qiagen RNeasy Mini kit protocol

1.2 Agilent RNA 6000 Nano Kit Guide protocol

1.3 SOP Tissue Samples Archiving

REVISION HISTORY:

Version	Date	Revision
1	01/30/2018	Updated materials, procedure, added Bioanalyzer, changed formatting

	Name	Signature	Date
Prepared by:	Maria Beery		
Approved by:	Irina Kusmartseva		