



STANDARD OPERATING PROCEDURE

DNA Extraction

OPPC-SOP-79

Prepared by:	Maria Beery and Stephen Selman	Original Effective Date:	05/05/2011
Revised by:	N/A	Version Effective Date:	08/15/2016
Reviewed by:	Maria Beery	Reviewed Date:	04/15/2021
Approved by:	Irina Kusmartseva	Approved Date:	05/06/2021

Current Version:	79.1
No. of Versions:	2
No. Pages:	4
Associated Forms:	N/A

Network for Pancreatic Organ Donation with Diabetes (nPOD)

BMSB Room J586
P.O. BOX 100275
Gainesville, FL 32610

JDRF nPOD Standard Operating Procedure	
SOP Number and Version: 79.1 Supersedes: 79.0	DNA EXTRACTION
Original Effective Date: 05/05/11	
Version Effective Date: 08/15/16	Page 2 of 4

DNA 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). Use chemical and physical safety precautions when working with paraformaldehyde and sharps, respectively.
- Buffer AL and AW1 contain guanidine hydrochloride, which can form highly reactive compounds when combined with bleach. Clean affected areas or spills with laboratory detergent and water, then 1% (v/v) sodium hypochlorite.
- PURPOSE:** The purpose of this Standard Operating Procedure (SOP) is to outline procedures for extracting high quality genomic DNA from nPOD samples.
- SCOPE:** This SOP will be applied to the extraction of DNA from fresh or snap frozen tissue, OCT-embedded frozen blocks, and formalin-fixed, paraffin-embedded blocks.
- RESPONSIBILITIES:** 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. They are responsible for following clinical laboratory and tissue banking best practices.
- EQUIPMENT & MATERIALS:** The materials, equipment and forms in the following list are recommendations only and alternative products may be substituted for the site specific task or procedure.
- DNeasy Blood & Tissue Kit (Qiagen, Cat. No. 69504)
 - Disposable Scalpel, #11 (FisherSci, Cat. No. NC0134996)
 - Microtube 1.5ml (VWR, Cat. No. 10025-726)
 - Microcentrifuge
 - Microcentrifuge tube racks
 - Vortex mixer
 - Hybridization oven or incubator, 56°C
 - 1x Tris-EDTA, DNase/RNase- free (pH 8.0, 10 mM Tris-HCl, 1 mM EDTA) (Fisher Cat. No. BP2473)
 - 100 % Ethanol (Molecular Biology Grade)
 - Xylene
 - Absorbent towels
 - Spectrophotometer (BioTek Epoch)
 - Take3 Micro-volume plate (BioTek)
 - Micropipettes and filter pipette tips (10µl, 200µl, 1000µl)
 - Nitrile gloves
 - Safety glasses
 - Disposable laboratory coats

JDRF nPOD Standard Operating Procedure		
SOP Number and Version: 79.1	Supersedes: 79.0	DNA EXTRACTION
Original Effective Date: 05/05/11		
Version Effective Date: 08/15/16		Page 3 of 4

PROCEDURE:

1.0 DNA Extraction from Tissue

- 1.1 Place tissue in a 1.5 ml microcentrifuge tube.
 - 1.1.1 Use 12-15 mg spleen or 25 mg pancreas.
 - 1.1.2 Mince further with sterile scissors or scalpel to aid digestion.
- 1.2 Add 180 µl Buffer ATL.
- 1.3 Add 20 µl proteinase K and mix thoroughly by vortexing.
- 1.4 Incubate at 56°C in a hybridization oven with moderate rotation until tissue is completely lysed.
 - 1.4.1 Vortex well every 30 minutes.
 - 1.4.2 Check level of digestion at 2 hours. If digestion is incomplete, continue 1-2 more hours.
- 1.5 Vortex 15 seconds, then add 200 µl Buffer AL and vortex thoroughly.
- 1.6 Add 200 µl 100% ethanol and vortex again.
- 1.7 Pipette the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube.
- 1.8 Centrifuge at ≥6000 x g for 1 minute. Discard flow-through and collection tube.
- 1.9 Place the DNeasy Mini spin column in a new 2 ml collection tube and add 500 µl Buffer AW1 to column.
- 1.10 Centrifuge for 1 minute at ≥6000 x g. Discard flow-through and collection tube.
- 1.11 Place the DNeasy column in a new 2 ml collection tube and add 500 µl Buffer AW2.
- 1.12 Centrifuge for 3 minutes at 20,000 x g to dry the DNeasy membrane. Discard flow-through and collection tube.
- 1.13 Place DNeasy Mini spin column in a new 1.5 ml microcentrifuge tube and add 100 µl 1x Tris-EDTA buffer to the center of the spin column membrane.
- 1.14 Incubate at room temperature for 5 minutes and then centrifuge for 1 minute at ≥6000 x g to elute DNA.
- 1.15 Proceed to section 4 for quantification and storage.

2.0 DNA Extraction from OCT Blocks

- 2.1 Section several thick sections of tissue under DNase-free conditions and place in microfuge tube. Proceed to step 1.2 above.

3.0 DNA Extraction from Formalin-fixed Paraffin-embedded Tissue Sections

- 3.1 Dissect a small amount of tissue with paraffin from block (0.3 x 0.3 x 0.3 cm³) using sterile disposable scalpel. Cut the tissue into 5-6 equal pieces and place into a microcentrifuge tube.
- 3.2 Add 1200 µl of xylene and incubate at room temperature for 2 minutes.
- 3.3 Centrifuge at 20,000 x g for 5 minutes.
- 3.4 Aspirate off the xylene and place tissue pieces on absorbent towels to dry.
- 3.5 Weigh the tissue.
 - 3.5.1 Use 7.5-12 mg of tissue for each preparation.
- 3.6 Finely mince the tissue with sterile scissors or scalpel to aid in digestion.
- 3.7 Place all minced tissue in microcentrifuge tube and repeat step 6.3.2.
- 3.8 Aspirate off the xylene and wash the tissue in 1200 µl of 100% ethanol. Spin at 20,000 x g for 5 minutes and aspirate ethanol.

JDRF nPOD Standard Operating Procedure		
SOP Number and Version: 79.1	Supersedes: 79.0	DNA EXTRACTION
Original Effective Date: 05/05/11		
Version Effective Date: 08/15/16		Page 4 of 4

- 3.9 Wash a second time with 1200 µl of 100% ethanol. Spin at 20,000 x g for 5 minutes and aspirate ethanol.
- 3.10 Incubate the open microcentrifuge tube at 37°C for 15 minutes until the ethanol has evaporated.
- 3.11 Re-suspend the tissue pellet in 180 µl ATL buffer.
- 3.12 Add 20 µl of proteinase K, mix by vortexing, and incubate in the hybridization oven with rotation at 56°C overnight until completely digested. Vortex occasionally during incubation.
- 3.13 Proceed to step 1.5.

4.0 Quantification and Quality Analysis

- 4.1 Measure optical density at 260 nm wavelength with a spectrophotometer and micro-volume plate, using 2µl per sample per microspot. Determine the A260/A280 nm wavelength ratio, which should be 1.8 – 2.0 for high quality DNA preparations.
- 4.2 Save all raw data. Import into the DNA Calculator Excel file to show A260/A280 ratio, stock DNA concentration (ng/µl), extraction volume (µl), and total DNA yield (ng).
- 4.3 Adjust DNA concentration to end-user specifications using 1x Tris-EDTA buffer and aliquot stock to avoid multiple thaw/freeze cycles.
 - 4.3.1 Expected yield from 10-15 mg spleen is 10-30 µg DNA.
- 4.4 Freeze DNA and store at -80°C according to SOP Tissue Sample Archiving.

REFERENCES:

1.0 Related Documents and Procedure

- 1.1 [Qiagen DNeasy Blood & Tissue Kit protocol](#)
- 1.2 [Biotek Epoch and Take3 Plate Manuals](#)
- 1.3 SOP Tissue Sample Archiving

REVISION HISTORY

Version	Date	Revision
1	07/28/2016	Updated materials, reagents, procedure, and quantification

	Name	Signature	Date
Prepared by:	Maria Beery and Stephen Selman		07/28/2016
Approved by:	Irina Kusmartseva		08/15/2016