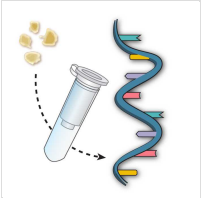

Jan 10, 2022

Pancreatic Islet RNA Extraction

Islet and Pancreas Analysis Core[1]

[1]Vanderbilt Diabetes Research Center

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Vanderbilt Diabetes Research Center

Islet and Pancreas Analysis Core
Vanderbilt Diabetes Research Center

This SOP defines the methods used by the Vanderbilt Diabetes Center Islet and Pancreas Analysis (IPA) Core for RNA extraction of pancreatic islets isolated from mouse or human tissue.

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Reagents and supplies:

- P-200 Aerosol Resistant Tips (ART) (Fisher 212361)
- P-1000 Aerosol Resistant Tips (ART) (Fisher P-2079E)
- RNase-free microcentrifuge tubes (Ambion NC9302929)
- PBS (Gibco 14190144)
- RNase ZAP! (Ambion AM9780)
- 100% ACS grade ETOH
- P200 Micropipette (Eppendorf Research Plus or similar)
- P1000 Micropipette (Eppendorf Research Plus or similar)

Kits:

- RNAqueous Micro Kit with DNase kit (Ambion AM-1931)
  Contains the following components:
  - Micro Filter Cartridges
  - Micro Tubes
  - Wash Solution 1
  - Wash Solution 2/3
  - Lysis Solution
  - Elution Solution

  DNase Kit:
  - LCM Additive
  - DNase
  - DNase Buffer
  - DNase Inactivation Reagent

Equipment:

- Centrifuge with swinging bucket rotor
- Vortexer
- Heat block with microcentrifuge tube adapter
- -80°C freezer
- -20°C freezer
- Microcentrifuge
- Water or bead bath set to 37°C

Prepare all working surfaces and equipment by spraying generously with RNase Zap!

Reagent preparation

1 **RNAqueous Kit** – Can be stored at **4 °C** up to 6 months.

1.1 Add 10.5 mL **100% ETOH** to *Wash Solution 1* bottle. Mark and date the bottle.

1.2 Add 22.4 mL **100% ETOH** to *Wash Solution 2/3* bottle. Mark and date the

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bottle.

2 **DNase Kit** – Can be stored at **-20 °C** up to 6 months.

2.1 Discard *LCM Additive* from DNase Kit.

RNA extraction procedure

3 Pick at least 75 islets into an RNase-free microcentrifuge tube. **Islets should be free of acinar tissue and other debris.**

For more information on picking islets with regard to size, refer to step 10 of the Static Incubation protocol:

Static Incubation of Pancreatic Islets
by Islet and Pancreas Analysis Core, Vanderbilt Diabetes Research Center

PREVIEW RUN

4 Centrifuge islets for **00:03:00** at **200 rcf, 4°C** .

5 Remove supernatant and add 1 mL cold **RNase-free PBS.**

6 Repeat steps 4-5 twice, for a total of 3 PBS washes.

7 Remove supernatant, add 200 mL **Lysis Solution**, and vortex to mix.

At this point, lysates can be stored at **-80 °C** for future RNA extraction. Islets can also be stored dry, but it is recommended by the kit protocol to freeze in Lysis Solution.

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8 Turn heat block to **75 °C** .

Pipet total required volume of **Elution Solution** for all samples into a microcentrifuge tube, cap tightly, and place in heat block. Use the table below to calculate volume needed per sample (each elution will be performed in two steps).

A	B	C	D	E	F
Sample IEQ	75 islets	100 islets	150 islets	200 islets	250+ islets
Elution steps	10 µL + 10 µL	15 µL + 10 µL	15 µL + 15µL	20 µL + 15 µL	20 µL + 20 µL
Total Elution Solution (µL)	20	25	30	35	40
Total samples					
Total Elution Solution for all samples (µL)					

Table 1: Recommended elution volumes. Copy and paste all cells above into an Excel sheet, then enter values into cells B4–F4. The total volume of Elution Solution required will be automatically returned in cell B6.

9 Remove DNase kit from -20°C to thaw at **Room temperature** .

10 Add 100 µL **100% ETOH** to each lysate and vortex to mix. If lysates were previously frozen, continue vortexing until thawed.

11 Prepare a Micro Filter Cartridge Assembly for each sample: insert a cartridge into a round-bottom microcentrifuge tube, and label both pieces.

12 Pipet 150 µL of lysate/ETOH mixture into the cartridge. Centrifuge for **00:00:10** at **18400 rcf** to pass the lysate through the cartridge and bind the RNA to the filter.

13 Repeat step 12 for the final 150 µL of lysate.

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14 Add 180 µL *Wash Solution 1* to the cartridge. Centrifuge for **00:00:10** at **18400 rcf** .

15 Remove cartridge from tube and discard contents. Replace cartridge back into tube.

16 Add 180 µL *Wash Solution 2/3* to the cartridge. Centrifuge for **00:00:10** at **18400 rcf** .

17 Repeat step 16 for a total of two washes with *Wash Solution 2/3*.

18 Centrifuge for **00:01:00** at **21300 rcf** to remove any residual liquid from the filter.

19 Transfer cartridge to a new, prelabeled round-bottom microcentrifuge tube. The schematic below illustrates steps 20-22.

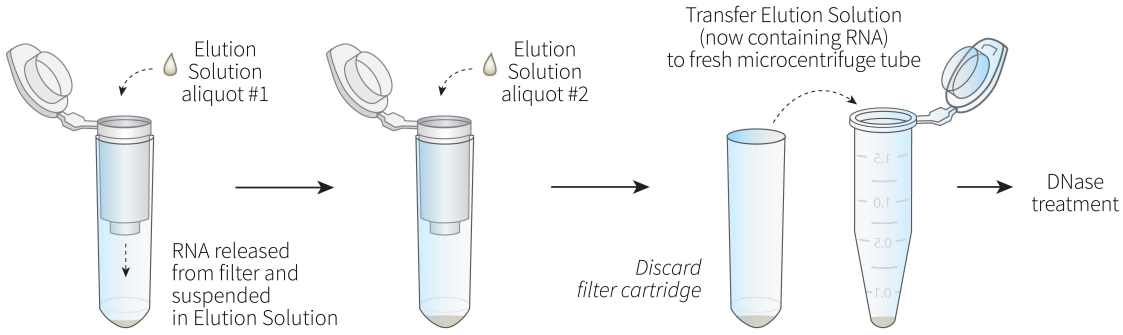

Figure 1: RNA elution is performed in two steps, then RNA is transferred to a fresh microcentrifuge tube for DNase treatment.

20 Add the first volume of pre-heated **Elution Solution** to the cartridge filter. Centrifuge for **00:00:30** at **18400 rcf** .

21 Repeat step 20 with the second aliquot of **Elution Solution.** Remove and discard filter cartridge.

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22 Label an RNase-free microcentrifuge tube and transfer the Elution Solution (now containing RNA) from the round-bottom microcentrifuge tube to the RNase-free tube.

DNase treatment procedure

23 Add 5 µL **DNase Buffer** and 1 µL **DNase** to each RNA sample. Vortex gently to mix.

24 Incubate samples in **37 °C** water bath for **00:20:00** .

25 Add 5 µL **Inactivation Reagent** to each sample, and vortex gently to mix.

26 Incubate samples at **25 °C** for **00:02:00** , vortexing gently after 1 minute of incubation, and then again after the incubation is complete.

27 Centrifuge samples for **00:01:30** at **21300 rcf** .

28 Being careful not to disturb the pellet, pipet the supernatant from each sample into a new, prelabeled RNase-free tube.

Measure RNA integrity and concentration

29 Assess the quality and concentration of RNA using the method of your choice. The IPA Core uses the services of the Vanderbilt University Medical Center VANTAGE Core.

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