



Sampling of Human Islets for Quality Control Purposes

James Lyon¹, Patrick MacDonald¹, Jocelyn Manning Fox¹

¹University of Alberta

dx.doi.org/10.17504/protocols.io.sizecf6



Jocelyn Manning Fox

University of Alberta



PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
Sodium Citrate Dihydrate	S279	Fisher Scientific
Sodium Chloride	BP358	Fisher Scientific
Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA)	ED2SS	Sigma Aldrich
Phosphate Buffered Saline (PBS)	BP24384	Fisher Scientific
Z-fix	NC9378601	Fisher Scientific
ART® 1000G Self-Sealing Barrier Pipet Tips Molecular BioProducts	53509-504	VWR international Ltd
ART® 200G Self-Sealing Barrier Pipet Tips Molecular BioProducts	53509-502	VWR international Ltd
95% Ethyl Alcohol	HC13001GL	Fisher Scientific
Acetic Acid Glacial ACS Grade ≥ 99.7%	LC101003	Fisher Scientific
Hydrochloric acid - concentrated	A149C4	Fisher Scientific

Islet Suspension

- 1 Suspend the islet preparation in a known volume of culture media (typically 50ml x2 for 100ml total volume). See [Human Islet Isolation Media Preparation](#) for CMRL preparation
Using a 25ml pipette and pipette aid, completely suspend the islet preparation.

Sampling - Insulin and DNA samples

- 2 Using a pipette with a wide bore ART® 200G Self-Sealing Barrier Pipet Tip, remove 4x 50µl samples from the islet suspension and transfer each sample to a 5ml polypropylene tube for DNA and insulin samples. See steps 5 & 6.

Sampling - Islet Equivalent counts

- 3 Using a pipette with a wide bore ART® 200G Self-Sealing Barrier Pipet Tip, remove one 50µl sample from the islet suspension and transfer the sample to a petri dish for an islet equivalent count performed twice on the same sample (Refer to [Human Islet Quantification and Purity Assessment](#) protocol.)

Sampling - Islet for Immunohistochemistry

- 4 Using a pipette with an ART® 1000G Self-Sealing Barrier Pipet Tip, remove one 500µl sample from the islet suspension and transfer to a 5ml polypropylene tube for histology. See step 7.

DNA samples

- 5 Add 4.5ml citrate buffer to each of 2 of the above samples step 2.
Centrifuge at 1500 rpm for 5 min.

⌚ 00:05:00

Using an aspirating pipette and a pipette tip remove all the resulting supernatant from the pelleted islets.

Label the sample tube with the internal identifier number (Rxxx), sample number (1 or 2), and sample date. Cap and store the sample tubes at 4°C until dsDNA assay.

Citrate Buffer - 1 Litre

To 800ml of Milli-Q water add the following reagents and allow to mix into solution. The citric acid will not completely go into solution until the pH is set to 7.4

- Sodium Citrate Dihydrate 3.15 g
- Sodium Chloride 8.77 g
- Disodium EDTA 1.01 g

Bring to volume with Milli-Q water and set the pH to 7.4.

Aliquot the Citrate buffer into 50ml conical tubes and store at -20°C. Thaw the buffer as needed and store at 4°C.

Insulin samples

- 6 Add 950µl of Acid/EtOH solution to each of the remaining two samples (step 2).

Label these sample tubes with the internal identifier number (Rxxx), sample number (a or b), and sample date.

Cap and store the sample tubes at -20°C to await insulin assay.

Acid/Ethanol solution

Add the following reagents in a sealed glass bottle. Store at 4°C prior to use.

- 150ml 95% ethanol
- 47ml acetic acid
- 3ml concentrated hydrochloric acid

Samples for histology

- 7 Add 4.5ml PBS to the 500µl sample (step 4) and allow the suspension to settle to a pellet for at least 5 minutes.

Remove the supernatant and add 500µl of Z-fix and transfer to a 1.5ml Eppendorf tube

Store tube @4°C overnight.

Remove the fixative and add 500µl PBS and store @4°C to await processing for histology

Submit to histology core for processing to blocks and slides.

Once the processed tissue is returned from histology store all blocks and slides in the histology library and record inventory on REDCap.

Library for Histology

- 8 Verify and record the sample type and location in the Histology Library record.

Sample Histology Library record:

Isolation Number	Sample									
	Pancreas Biopsy		Post-isolation		Pre-distribution		Pre-cryopreservation		Post-cryopreservation	
	Blocks	Slides	Blocks	slides	Blocks	slides	Blocks	slides	Blocks	slides
R001	1	4	1	4	1	4	1	4	1	4
R002	1	4	1	4	1	4	1	4	1	4
R003	1	4	1	4	1	4	1	4	1	4
R004	1	4	1	4	1	4	1	4	1	4
R005	1	4	1	4	1	4	1	4	1	4
R006	1	4	1	4	1	4	1	4	1	4
R007	1	4	1	4	1	4	1	4	1	4



This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

