Version 2

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Sampling of Human Islets for Quality Control Purposes V.2

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1 Works for me dx.doi.org/10.17504/protocols.io.bupbnvin

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ABSTRACT

This protocol described the sampling of human islets for quality control purposes, as performed by the Alberta Diabetes Institute IsletCore. http://www.bcell.org/adi-isletcore.html

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WHAT'S NEW

Minor edits for clarity.

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MATERIALS TEXT

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Sodium Citrate Dihydrate **Fisher Scientific Catalog #S279**

Sodium Chloride **Fisher Scientific Catalog #BP358**

Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) **Sigma Aldrich Catalog #ED2SS**

Phosphate Buffered Saline (PBS) **Fisher Scientific Catalog #BP24384**

Z-fix **Fisher Scientific Catalog #NC9378601**

ART® 1000G Self-Sealing Barrier Pipet Tips Molecular BioProducts **VWR international Ltd Catalog #53509-504**

ART® 200G Self-Sealing Barrier Pipet Tips Molecular BioProducts **VWR international Ltd Catalog #53509-502**

95% Ethyl Alcohol **Fisher Scientific Catalog #HC13001GL**

Acetic Acid Glacial ACS Grade ≥ 99.7% **Fisher Scientific Catalog #LC101003**

Hydrochloric acid - concentrated **Fisher Scientific Catalog #A149C4**

Solution Preparation

1 To **800 mL** 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

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

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** .

2 **Acid/Ethanol solution**

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

- **150 mL** 95% ethanol
- **47 mL** acetic acid
- **3 mL** concentrated hydrochloric acid

Islet Suspension

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3 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 by gently pipetting up and down.

Sampling - Islet Equivalent counts

4 Using a pipette with a wide bore ART® 200G Self-Sealing Barrier Pipet Tip, remove one **50 µl** sample from the centre of 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 - DNA and insulin samples

5 Using a pipette with a wide bore ART® 200G Self-Sealing Barrier Pipet Tip, remove 4x **50 µl** samples from the centre of the islet suspension and transfer each sample to a 5ml polypropylene tube for DNA (x2) and insulin samples (x2).

DNA samples

6 Add **4.5 mL** citrate buffer to each of the 2 samples indicated for DNA in step 5.
Centrifuge at **1500 rpm, 00:05:00** .

6.1 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.

Insulin samples

7 Add **950 µl** of Acid/Ethanol solution to each of 2 of the above samples (step 5).

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.

Histology Samples

8 Using a pipette with an ART® 1000G Self-Sealing Barrier Pipet Tip, remove one **500 µl** sample from the islet suspension and transfer to a 5ml polypropylene tube labelled with the internal identifier number (Rxxx) for histology.

Add **4.5 mL** PBS to the **500 µl** sample and allow the suspension to settle to a pellet for at least **00:05:00** .

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

Store tube at **4 °C** **Overnight** .

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

Embed fixed islet sample in low melt agarose and submit to histology core for processing to paraffin-embedded blocks and sections.

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

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