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Version 1

Sampling of Human Islets for Quality Control Purposes V.1

 Endocrinology

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Human Cell Atlas Metho...

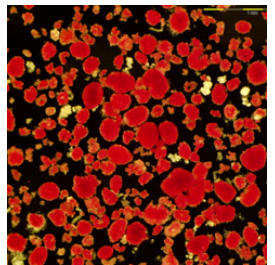
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Protocol status: Working

We use this protocol and it's working

Created: August 11, 2018

Last Modified: January 26, 2019

Protocol Integer ID: 14649

Keywords: human islets for quality control purpose, human islet

Materials

MATERIALS

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

 Sodium Chloride **Fisher Scientific Catalog #BP358**

 Ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA) **Merck MilliporeSigma (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 (Avantor) Catalog #53509-504**

 ART® 200G Self-Sealing Barrier Pipet Tips Molecular BioProducts **VWR International (Avantor) 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**

Troubleshooting



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 4× 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 950 μl 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 500 μl sample (step 4) and allow the suspension to settle to a pellet for at least 5 minutes.

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

Store tube @ 4°C overnight.

Remove the fixative and add 500 μl 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:

	Iso lati on Nu m be r	Sample									
		Pancrea s Biopsy		Post- isolati on		Pre- distrib ution		Pre- cryopr eserva tion		Post- cryopr eserva tion	
		Blocks	Sli de s	Block s	sli de s	Block s	sli de s	Blocks	slid es	Blocks	slides
	R0 01	1	4	1	4	1	4	1	4	1	4
	R0 02	1	4	1	4	1	4	1	4	1	4
	R0 03	1	4	1	4	1	4	1	4	1	4
	R0 04	1	4	1	4	1	4	1	4	1	4
	R0 05	1	4	1	4	1	4	1	4	1	4
	R0 06	1	4	1	4	1	4	1	4	1	4
	R0 07	1	4	1	4	1	4	1	4	1	4