Islet Sampling for Quality Control Assessment – Adenine and Pyridine Nucleotides

Purpose: To prepare samples of the final islet suspension for quality control assessment by HPLC prior to transplantation.

Cellular Parameters to be Assessed:	Acceptable Values
ATP/ADP An indicator of the cellular energy state that shows good correlation with initiation of apoptosis.	≥ 10
Adenylate Energy Charge (AEC) is an index for the amount of metabolically available energy in a cell in the form of adenosine phosphates. The inclusion of AMP allows for detection of metabolically deficient cells in which the ATP/ADP ratio may not have changed greatly. AEC = ATP + (0.5*ADP)/ATP+ADP+AMP	≥ 0.850
NADH/NAD$^+$ An indicator of the cellular/mitochondrial redox state that shows good correlation with initiation of apoptosis.	≤ 0.10
NADPH/NADP$^+$ An indicator of the cellular redox state that also shows correlation with beta cell insulin secretory capacity.	≥ 1.0

Tissue Samples: An aliquot of the **top layer** islet suspension containing approximately 1,500 IEQ.

Materials and Reagents:

- Sarstedt screw top microfuge tubes (1.5 mL, V bottom #72.692.005)
- 15 or 50 mL conical centrifuge tubes
- 30 mm grid lined petri dishes
- Micropipettors
- Swinging bucket centrifuge, micro-centrifuge
- Inverted microscope

Dithizone staining solution

- 0.1g dithizone, 10mL DMSO, 40mL 1x PBS
- Load into a 60mL syringe
- Place a 0.22µm syringe filter on the tip
- Store at room temp

EDTA-water (66mM)

- Add 13.3g EDTA (Dipotassium salt, Acros, #209741000) to 500mL HPLC grade water
- Adjust pH to 7.4
- Sterilize by autoclave or filtration (0.22µm)

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Phenol

- Melt 100g Phenol at 60ºC until clear
- Transfer to an opaque **glass** container
- Add 100mL EDTA-Water
- Tightly close the container
- Shake hard
- Let sit for 20 min at room temp
- Take off the upper phase (EDTA-water phase), dispose appropriately
- Add 100mL EDTA-water on top of the phenol
- Shake again and let sit for another 20 min. at room temp

PBS Wash/Extract Buffer

- Standard 1xPBS (no Ca+ and Mg++) made from 10x stock (Mediatech SH30258.02)
- Add EDTA (dipotassium salt) to a final concentration of 66 mM
- Adjust pH to 7.4
- Sterilize by autoclave or filtration (0.22 μm)
- Store at 4ºC
- Place on ice prior to use

PCI Solution

- Pipette 34mL Phenol, 24mL Chloroform and 1mL Isoamyl alcohol (3-Methyl-1-Butanol) together in an opaque glass container
- Add 100mL EDTA-water
- Shake the container
- Let sit until the phases are separated
- Store at 4ºC in the dark
- Place on ice prior to use
- **Upper phase is the EDTA-water**, bottom phase is the PCI

Ether:

- Pipette 100mL ethyl ether into a glass container
- Add 100mL EDTA-water
- Shake the container
- Let sit until the phases are separated
- Add 100mL EDTA-water
- Shake the container
- Let sit until the phases are separated
- **Upper phase is the Ether**, bottom phase is the EDTA-water

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Procedure:

1. Triplicate aliquots of islets are counted by dithizone staining and manual observation using an inverted microscope and eyepiece reticle. Islet quantity is determined by the standard conversion to islet equivalents (IEQ) method.

2. Tube labeling:
   a. Isolation Code (may be center specific or ICR designated)
   b. Time in culture post isolation (e.g. T0, or T24)
   c. Date
   d. Initials of person making the aliquots

3. Samples are then aliquoted in the following manner:

   a. ATP samples (3x 500 IEQ each).
   b. After determining the concentration of islets per unit volume of tissue culture media, 1500 islet equivalents are placed into a conical centrifuge tube (e.g. 15 or 50 mL).
   c. Centrifuge islets for 1 minute at 1000 rpm without braking in a swinging bucket centrifuge. Discard supernatant by pipetting (do not decant).
   d. Resuspend islets in 3 mL of ice cold PBS + 66 mM EDTA.
   e. Aliquot 1mL each into 3 microfuge tubes.
   f. Pulse spin islets in a microcentrifuge to gently pellet the islets. Remove PBS + 66 mM EDTA solution by pipetting.
   g. Add 300 µL ice cold PBS + 66 mM EDTA per tube.
   h. Add 1 mL cold Phenol Chloroform Isoamyl Alcohol to each tube. *BE SURE THAT YOU ARE ADDING THE ORGANIC PHASE!*
   i. Vortex tubes until no intact islets are visible (approx 30 sec each).
   j. Snap freeze in liquid nitrogen.
   k. Store at -80°C

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References:

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