Islet Sampling for Protein, RNA, MDA, and GpX Assessment

Purpose: To prepare samples of the islets after shipment for MDA, GPX, Protein, and RNA. Islet requirement is a minimum of 8000 IEQ.

Reagents and Materials:

- Dithizone staining solution (0.1 gr dithizone, 10 ml DMSO, 40 ml 1x PBS)
- PBS (cold)
- TRIzol reagent (Invitrogen)
- Sarstedt screw top microfuge tubes
- 30 mm grid lined petri dishes
- Liquid nitrogen

Protocol:

1. After removal of the islets from the transport container an aliquot is counted by dithizone staining and manual observation using an inverted microscope and eyepiece reticle.

2. Label Sarstedt screw top microfuge tubes: Each tube should contain the isolation code, sample type (ie RNA), islet number in tube (ie 1000 IEQ) , time point of sample (ie 18 hours post isolation), date and initials

3. Samples are then aliquoted in the following manner:

    a. **RNA samples for RT-PCR** (1-3 samples)
        - At a minimum RNA samples should contain a minimum of 2000 IEQ, up to 10000 IEQ. Determine volume of islet suspension needed and place in a 15 or 50 mL centrifuge tube(s) as needed.
        - Centrifuge in a clinical centrifuge at 1000 rpm for 1 minute with no brake. Take off media by aspiration with a pipet, taking care not to disturb the loose pellet.
        - Resuspend at a concentration of 1mL PBS per # of RNA samples.
        - Aliquot 1mL per pre-labeled microfuge tube.
        - Pulse spin islets a microfuge to gently pellet the islets. Remove the supernatant taking care to remove as much PBS as possible.
        - Add 1mL trizol reagent to each tube.
        - Vortex tubes until no intact cells are visible (approx 10 sec each).
        - Store at -80°C

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b. **Protein samples** (1-3 samples)
- At a minimum protein samples should contain 3000 IEQ, with an ideal sample of 5000 IEQ. Determine volume of islet suspension needed and place in a 15 or 50 mL centrifuge tube(s) as needed.
- Centrifuge islets for 1 minute at 1000 rpm without braking in a clinical centrifuge. Take off supernatant.
- Resuspend at 1 mL ice cold PBS per number of protein of samples.
- Aliquot 1mL per Sarstedt screwtop microfuge tube.
- Pulse spin islets a microfuge to gently pellet the islets.
- Wash islet pellets with 1 mL cold PBS, pulse spin to pellet islets.
- Take off supernatant. Take care to remove as much PBS as possible, leaving an essentially dry pellet.
- Snap freeze the islets in liquid nitrogen.
- Store at -80°C.

c. **MDA and GPX samples** (2-3 GPX, 2-3 MDA)
- MDA and GPX samples should contain 750-1500 IEQ per sample. Determine volume of islet suspension needed and place in a 15 or 50 mL centrifuge tube(s) as needed.
- Centrifuge islets for 1 minute at 1000 rpm without braking in a clinical centrifuge. Take off supernatant.
- Resuspend at 1 mL ice cold PBS per number of protein of samples.
- Aliquot 1mL per Sarstedt screwtop microfuge tube.
- Pulse spin islets a microfuge to gently pellet the islets.
- Wash islet pellets with 1 mL cold PBS, pulse spin to pellet islets.
- Take off supernatant. Take care to remove as much PBS as possible, leaving an essentially dry pellet.
- Snap freeze the islets in liquid nitrogen.
- Store at -80°C.

4. All samples are then shipped to the UW-Madison Islet Core Facility on dry ice.

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