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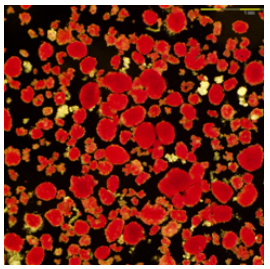
Human Islet Purification - COBE setup



Endocrinology

In 2 collections

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Human Cell Atlas Metho... CIRTNR2FIC 1 more workspace

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OPEN ACCESS

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Protocol status: Working
We use this protocol and it's working

Created: August 09, 2018

Last Modified: January 25, 2019

Protocol Integer ID: 14627

Guidelines

Refer to **Human Islet Isolation Media Preparation** protocol for solutions used in the current protocol.
Perform all procedures aseptically.
Refer to included photos for visualization of set up.

Materials

MATERIALS

Cell Processing Set **TerumoBCT Catalog #**90819

COBE 2991 Cell Processor **TerumoBCT Catalog #**2991

Purge

1 Turn on COBE 2991 processors and purge as follows:
- Set dials; centrifuge speed to 3000 rpm, super out rate to 450 mL/min and super out volume to 600 mL.
- Install foam ring and plastic shim in to COBE.
- Press START/SPIN, when rpm are at 3000, press SUPER OUT.
- Once alarm sounds press STOP/RESET.
- Allow COBE 2991 bowl to drain before placing cell processing bag in processor.
- Set dials; centrifuge speed to 1500 rpm, and super out rate to 0 mL/min.

Install COBE tubing/bag set

2
- Open centrifuge latch by rotating clockwise, remove metal seal weight, and open sliding covers.
- Remove locking cover by rotating counter clockwise while holding up locking pin.
- Remove white alignment blocks, small plastic cover, and foam insert.
- Feed sterile cell processing bag through centre hole of locking cover.
- Install bag in centrifuge bowl by aligning holes in bag over studs on bowl. Spread bag out evenly in attempt to eliminate creases.
- Place white alignment blocks with bevelled end facing up around center stem of bag. Place locking cover around blocks and over studs. Rotate cover clockwise until locking pin drops into place.
- Close sliding covers, place metal seal weight on hexagonal seal of cell processing bag and close centrifuge latch by rotating counter clockwise.
- Clamp all bag lines near manifold with haemostats, except for top left or right line depending on pump orientation.
- Support main bag line upright by placing all clamped lines through pinch valves on COBE 2991
- Place end of main line in Biosafety Cabinet, remove line cap and connect to end of COBE connector tubing.

IMG_6756.jpg IMG_6758.jpg

install gradient maker

3 Open sterilized gradient maker set and place sterilized stir bar in front beaker of gradient maker
Place assembled gradient maker set on top of magnetic stir plate.
Clamp tubing between beakers with haemostats.

Place section of the COBE connector tubing through Masterflex pump with open end of tubing facing adjacent COBE 2991, and close latch.

IMG_6757.jpg IMG_6756.jpg

Prepare tissue collection conical tubes

4 Open two packages of sterile 250 mL conical tubes and place into 250 mL racks. Label 250 mL conicals as numbers 1 to 12. If there is more than 1 purification run the conical tubes shall also be designated with a letter (E.g. A1 - A12, B1 - B12...).
Refer to **Human Islet Isolation Media Preparation** protocol.

- Fill conical 1 with 100 mL of wash 2 solution
- Fill conical 2 with 150 mL of wash 2 solution.
- Fill conicals 3 – 4 with 200 mL of wash 2 solution.
- Fill conicals 5 – 11 with 225 mL of wash 2 solution.
- Fill conical 12 with 100 mL of dilution 1 solution.
- Place conicals in refrigerator until use.