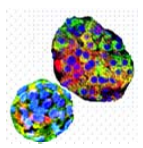


SOP



Title:	Mouse islet isolation				
Protocol #:	1.4	Submitted:	050510	Approved:	200610
Category:	Cyto	Author(s): ¹	MRU	Checked by:	AAH

Reagents:

1. DMEM (Cellgro catalog # 10-013-CV; 4.5g/L glucose)
2. RPMI
3. FBS (GIBCO, cat. no. 10270-106)
4. Collagenase (type V) for pancreatic islets Sigma
5. 0.25% trypsin/1 mM ethylenediaminetetraacetic acid (GIBCO, cat. no. 27250-018)
6. Streptomycin/ Penicillin
7. L-glutamine (GIBCO, cat. no. 35050-038)

Equipment

1. Hood for cell culture with vertical laminar flow and equipped with UV light for decontamination (PC2 certified)
2. Water bath with temperature control
3. Clinical centrifuge (no temperature control is needed)
4. Incubator with both temperature and gas composition controls
5. Inverted microscope with phase-
6. Culture flasks
7. Weighing balance
8. Surgical forceps (straight and curved)
9. Surgical scissors
10. Disposable Pipettes (ARS, cat. no. 537-503/200)

Reagent Setup

1. Weigh Collagenase: 3mg/ml (21 mg / 15-ml-conical tube): Use appropriate safety gear while weighing collagenase. You will need to filter this (using 0.22 μ m syringe filters) in DMEM medium prior to use.
 2. DMEM : Keep cold at 4°C *
 3. RPMI containing 10% FBS *: Pre-warm in 37°C water bath
- *Note: I generally prefer no antibiotics in the media, but these can be added if necessary. All antibiotics are stored in the freezers in each culture room.

Procedure:

1. Euthanize mice with compressed CO₂ followed by cervical dislocation to ensure no discomfort to the animals. Submerge in 70% EtOH (in 50ml conical tube) to disinfect. All of the following procedure, unless otherwise specified, is performed in aseptic conditions on ice.

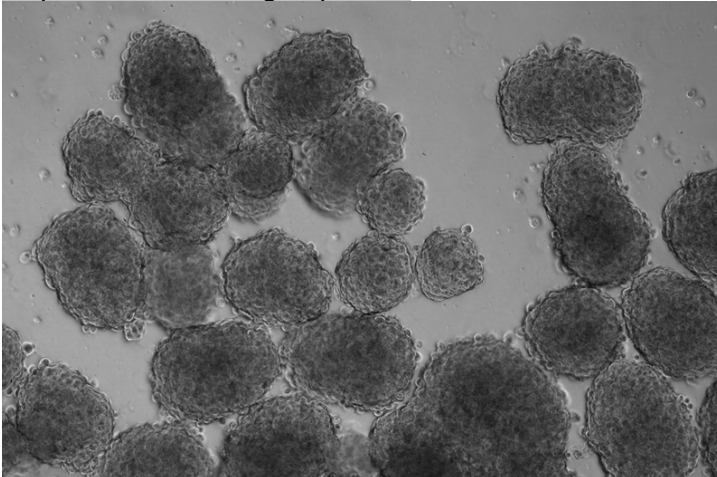
SOP

2. Open the mouse to expose the spleen.
3. Pull out the spleen and the majority (~70%) of the pancreas attached to the spleen. (You may also pull out the mesenteric mass of the pancreas at this time).
4. Remove pancreas and put them directly into 20ml DMEM, in a 15 ml conical tube. Pool the pancreas from 3-6 mice. At this time make sure that there is no fat attached to the excised tissue (fat usually floats on media) and wash the tissue with cold DMEM medium for a couple of times.
5. Remove as much media as possible and transfer pancreas to a 10cm glass dish.
6. With scissors (or surgical blades) chop tissue to around 1mm³ pieces.
7. Transfer tissue to 7ml collagenase (3mg/ml) in a 15ml tube (this is generally enough for pancreas from 4-5 mice).
8. Seal tube with parafilm and shake vigorously in a 37°C water bath for about 3-5min, continuing to shake gently for another 3-5 minutes or so or until the tissue is digested (no large visible chunks).
9. Spin For 3 min at 150xg.
10. Wash 2 x in RPMI.
11. Plate cells in non-tissue culture treated flasks in RPMI (containing 10% FBS).
12. Incubate at 37°C for 3 days in RPMI (May need to Refeed inspect for exocrine and ductal cell attachment and death).
13. Spin down islet at 150xg for 3min.
14. Resuspend in CMRL 1066 + 10%FCS + Glutamine + penn/strep*.
15. Plate islets on tissue culture treated dishes and maintain cells in SC CMRL.

Anticipated results

N/A

Representative image / picture



Notes:

A word about Collagenase and trypsin: Collagenases are enzymes that break the peptide bonds in collagen. It requires Ca and Mg for its action and so it is important to prepare it in DMEM / media. Adherent cells produce integrins (proteins) that help cells to attach to the plate (and each other). Mg & Ca are

SOP

divalent cations that are essential to protein activity. Always keep in mind that if you want cells to adhere & grow, you want the cations. If you want to suspend them, you want to avoid/reduce the cations. Trypsin /EDTA is used to loosen cells from substrate so they can be resuspended (For example, when you want to prepare single cell suspension from your islet cultures for FACS). Trypsin is a protease that digests the adhesion proteins, which hold cells to the plate. EDTA is a chelator that mops up divalent cations that are cofactors for protein activity. By removing magnesium and calcium, cells lose contact with each other, there is no adhesion and they “round up” in solution.