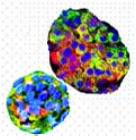

SOP

Title:	**Static stimulation of Islet**				
Protocol #:	1.4	Submitted:	050510	Approved:	200610
Category:	CC	Author(s):[1]	MRU,MVJ	Checked by:	AAH

Reagents:

1. FBS (GIBCO, cat. no. 10270-106)
2. RPMI 1640 medium
3. BSA (Sigma)
4. Glucose
5. PBS

Equipment

1. Stereo microscope
2. 50 mm glass peri plates
3. Pipette (20 - 200µl)

Reagent Setup

1. Pre-warm: 20 mM glucose, 2.8 mM glucose and KCl solutions

Procedure:

Exposure to incretins

1. Islets or *in vitro* derived islet-like cell clusters (ICCs) should be taken into 50 mm glass peri plates and handpicked using stereo microscope. During handpicking, choose clusters with a size of ~150 um diameter or smaller as these contain most viable cells and are easily picked up with a fine drawn capillary / pipette (even with a P-200).

2. Handpick 200 islet equivalents (IEQs) in each eppendorf tube.

3. Incubated these in RPMI 1640 medium without (control) or with the addition of either incretins (100 nM GLP-1 and 5 µm CCK) or NIC (10mM) for a period of 6 h.

4. After incubation of ICCs for 6 h, their ability to secrete insulin in response to glucose can be examined by *in vitro* static stimulation.

Static stimulation

1. Briefly, ICCs are washed with PBS (1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 26.7 mM $NaHCO_3$, and 20 mM HEPES) containing 0.2% BSA (Sigma) and exposed in quadruplets to this basal (PBS and 2.8 mM

glucose) or stimulated (PBS and 20 mM glucose) buffer for 1 h at 37°C.

2. At the end of the hour, pellet down cells by allowing them to settle down and gently remove and store the supernatant for insulin released.

3. The insulin content of the ICCs is estimated after sonicating the ICC pellet in 200µl acid/ethanol (Prepared by adding 18 ml 10M HCl / liter 70% ethanol: Keep chilled in the freezer room at -20°C). Insulin is extracted by incubating the cells overnight in acid ethanol at 4 ^{o}C or stored at -80°C till assay. Insulin concentrations are measured by ELISA (Linco / Alpco) of the lysates.

Anticipated results
N/A

Representative image / picture
N/A

References:
N/A