VERSION 2

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 We use this protocol and it's working

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Static Glucose-stimulated Insulin Secretion (GSIS) Protocol - Human Islets V.2

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MATERIALS

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- BSA Sigma Aldrich Catalog #A7906
- Sodium bicarbonate Sigma Aldrich Catalog #S5761
- HEPES Fisher Scientific Catalog #BP310-500
- Sodium Chloride Fisher Scientific Catalog #BP358-212
- Penicillin-Streptomycin Gibco - Thermo Fisher Catalog #15140122
- FBS (Canadian Origin) Gibco - Thermo Fischer Catalog #12483-020
- Potassium Chloride Sigma Aldrich Catalog #P9541
- Calcium Chloride Sigma Aldrich Catalog #C4904
- Magnesium Chloride Hexahydrate Emd Millipore Catalog #MX0045
- DMEM Gibco - Thermo Fischer Catalog #11885
- STELLUX® Chemi Human Insulin ELISA Jumbo Alpco Catalog #80-INSHU-CH10

Day Before Experiment

- 1 Pick human islets of similar size and shape into Human Islet Culture Media until as close as possible to 100% purity.

Human Islet Culture Media

500ml DME M (5mM glucose)	Gibco 11885
50ml FBS Canadian Origin	Gibco 12483 -020
5ml Pen/strep	Gibco 15140 -122

- 2 Culture islets overnight in incubator at 37°C, 5% CO₂.

Day of experiment: Solution Preparation

- 3 Prepare KRBH solution as follows:

	mM Final	per 100mL total
NaCl	115	5.75 mL (2M)
KCl	5	0.5mL (1M)
NaHCO₃	24	0.2g
CaCl₂	2.5	0.25 mL (1M)
MgCl₂	1	0.1mL (1M)
HEPES	10	1mL (1M)
BSA	0.1% w/v	0.1g

Warm KRBH solution to 37°C in incubator 37°C, 5% CO₂ (Approximately 30 min). Once solution is warmed, pH KRBH solution to 7.4 with NaOH and bring to volume. KRBH Solution should be kept in incubator throughout the experiment.

Add glucose and/or additional treatments as required. eg:

	per 50mL total	from 1M stock	from powder
	1mM	50µL	0.009 g
	2.8mM	140µL	0.025 g
	10mM	500µL	0.090 g
	16.7mM	835µL	0.150 g

Acid Ethanol - ensure acid ethanol is available

95% Ethanol	150mL
Acetic Acid	47mL
Concentrated HCL	3mL

Experimental Protocol

- 4 Pick islets into 35mM non-tissue cultured coated (NTCC) dish and 'wash' islets with 2mL of appropriate low glucose (1mM or 2.8mM) KRBH.
- 5 Pick islets into new 35mM NTCC dish and pre-incubate in 2mL of appropriate low glucose (1mM or 2.8mM) KRBH at 37°C, 5% CO₂ for 1 hour.

2 mL low glucose
KRBH

37 °C

01:00:00

- 6 Transfer islets into a new 35mM NTCC dish, add 2ml low glucose KRBH and pre-incubate for 1 hour at 37°C, 5% CO₂.

2 mL low glucose
KRBH

37 °C

01:00:00

- 7 Pick 15 islets/tube into labelled eppendorf tubes. Each treatment group should be done in triplicate.

- For Baseline GSIS for Isletcore three groups.
- 1mM glucose to 10mM glucose
-
- 1mM glucose to 16.7mM glucose
-
- 2.8mM glucose to 16.7mM glucose
-

- 8 Add 500µL of low glucose KRBH, and incubate for 1 at 37°C, 5% CO₂. Leave tube lids open.

500 µL low glucose
KRBH

37 °C

01:00:00

- 9 Close lids, gently invert tubes, and spin at 1000 RPM in a benchtop centrifuge for 1 minute to pellet islets.

- 10 Collect 500µL of the supernatant, or as much as possible without disturbing the pellet. Store supernatant at -20°C until insulin assay.

-20 °C

- 11 Resuspend islets in 500µL of high glucose KRBH, and incubate for 1 hour at 37°C, 5% CO₂. Leave lids open.

500 µL high glucose
KRBH

37 °C

01:00:00

- 12** Close lids, gently invert tubes, and spin at 1000 RPM for 1 minute to pellet islets.
- 13** Collect 500µL of the supernatant, or as much as possible without disturbing the pellet. Store supernatant at -20°C until insulin assay.
-20 °C
- 14** Add 500µL of acid ethanol to the islets and vortex. Store tube at -20°C until insulin assay.
500 µL acid ethanol
-20 °C

ELISA

- 15** Samples are assayed using ALPCO Stellux Human Insulin ELISA kit (cat# 80-INSHU-CH10). Content samples are diluted with zero buffer 1:400. High glucose samples may need to be diluted with zero buffer 1:10.