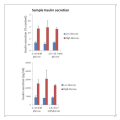

Static Glucose-stimulated Insulin Secretion (GSIS) Protocol - Human Islets

Version 1

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PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

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

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 DMEM (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% CO_2.

Day of experiment: Solution Preparation

3 **Prepare KRBH solution as follows:**

	mM Final	per 100mL total
NaCl	115	5.75mL (2M)
KCl	5	0.5mL (1M)
$NaHCO_3$	24	0.2g
$CaCl_2$	2.5	0.25mL (1M)
$MgCl_2$	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% CO_2 (Appoximately 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	0.009g
2.8mM	140µL	0.025g
10mM	500µL	0.090g
16.7mM	835µL	0.150g

Acid Ethanol - ensure acide 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% CO_2 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% CO_2.

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% CO_2. 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% CO_2. 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.