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 We use this protocol and it's working

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Static Glucose-stimulated Insulin Secretion (GSIS) Protocol: Mouse Islets

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ABSTRACT

Static glucose stimulated insulin secretion (GSIS) protocol for mouse islets.

MATERIALS

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- BSA **Sigma Aldrich Catalog #A7906**
- Sodium bicarbonate **Sigma Aldrich Catalog #S5761**
- HEPES **Fisher Scientific Catalog #BP310-500**
- Penicillin-Streptomycin **Gibco - Thermo Fischer Catalog #15140122**
- RPMI 1640 **Gibco - Thermo Fischer Catalog #11875**
- FBS (Canadian Origin) **Gibco - Thermo Fischer Catalog #12483-020**
- Sodium Chloride **Fisher Scientific Catalog #BP358-212**
- Potassium Chloride **Sigma Aldrich Catalog #P9541**
- Calcium Chloride **Sigma Aldrich Catalog #C4904**
- Magnesium Chloride Hexahydrate **Emd Millipore Catalog #MX0045**
- D-()-Glucose **Sigma Aldrich Catalog #G8270**
- STELLUX® Chemi Rodent Insulin ELISA Jumbo **Alpco Catalog #80-INSMR-CH10**

Day Before Secretion

1 Mouse islets isolated as described in [Mouse Islet Isolation](#) protocol.

2 Pick mouse islets into Mouse Islet Culture Media until as close as possible to 100% purity.

Mouse Islet Culture Media

500ml RPMI 1640 (11.1mM glucose)	Gibco 11875-119
50ml FBS Canadian Origin	Gibco 12483-020
5ml Pen/strep	Gibco 15140-122

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

Solution Preparation

4 Prepare solutions as follows:

A	B	C
	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

KRBH is made fresh on the day of the experiment

Mix chemicals from above table in milliQ water (approximately 80 mL). 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 (100 mL). KRBH Solution should be kept in incubator throughout the experiment.

Add glucose and/or additional treatments as required.

A	B	C
per 50mL total	from 1M stock	from powder
2.8mM	140μL	0.025g
16.7mM	835μL	0.150g

1M glucose stock should be made fresh on day of experiment

Acid Ethanol:

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

This solution can be made in advance

Experimental Protocol

- Pick islets into 35mm non-tissue cultured coated (NTCC) dish and 'wash' islets with 2 mL of KRBH with low glucose (2.8mM).
- Pick islets into new 35mm NTCC dish in 2 mL of low glucose KRBH and pre-incubate in incubator at 37 °C 5 % CO₂ for 01:00:00 . 1h
- Transfer islets into a new 35mm NTCC dish and add 2 mL of low glucose (2.8mM) KRBH and pre-incubate for 01:00:00 at 37 °C , 5 % CO₂. 1h

- 8 Pick 15 islets into eppendorf tubes. Each treatment group should be done in triplicate.
- Control group is typically 2.8mM glucose for low glucose and 16.7mM glucose for high glucose
 - Number of islets can be increased/decreased depending on ELISA kit sensitivity
- 9 Gently add 500 μL of low glucose KRBH to the islets, and incubate for 01:00:00 at 37 $^{\circ}\text{C}$, 5 % CO_2 . Leave tube lids open. 1h
- 10 Close lids, gently invert tubes, and centrifuge at 1000 rpm, 00:01:00 to pellet islets. 1m
- 11 Collect as much of the 500 μL supernatant as possible without disturbing the pellet. Store supernatant at -20 $^{\circ}\text{C}$ until insulin assay.
- 12 Gently add 500 μL of high glucose (16.7mM) KRBH to islets, and incubate for 01:00:00 with conditions at 37 $^{\circ}\text{C}$, 5 % CO_2 . Leave lids open. 1h
- 13 Close lids, gently invert tubes, and centrifuge at 1000 rpm, 00:01:00 to pellet islets. 1m
- 14 Collect as much of the 500 μL supernatant as possible without disturbing the pellet. Store supernatant at -20 $^{\circ}\text{C}$ until insulin assay. -20 $^{\circ}\text{C}$
- 15 Add 500 μL of acid ethanol to the islets. Store tube at -20 $^{\circ}\text{C}$ until insulin assay.

ELISA

- 16** Samples are assayed using ALPCO Stellux Rodent Insulin ELISA kit (Cat # 80-INSMR-CH10). Content samples are diluted with zero buffer 1:400.