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

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1 Works for me dx.doi.org/10.17504/protocols.io.sp7edrn

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ABSTRACT

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

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MATERIALS TEXT

MATERIALS

BSA **Sigma**

Aldrich Catalog #A7906

Sodium bicarbonate **Sigma**

Aldrich Catalog #S5761

HEPES **Fisher**

Scientific Catalog #BP310-500

Penicillin-Streptomycin **Gibco - Thermo**

Fisher 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% CO_2.

Solution Preparation

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4 **Prepare solutions as follows:**

A	B	C
	mM Final	per 100mL total
NaCl	115	5.75mL (2M)
KCl	5	0.5mL (1M)
NaHCO3	24	0.2g
CaCl2	2.5	0.25mL (1M)
MgCl2	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% CO_2 (Approximately 30 min). Once solution is warmed, pH KRBH solution to pH **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

5 Pick islets into 35mm non-tissue cultured coated (NTCC) dish and 'wash' islets with **2 mL** of KRBH with low glucose (2.8mM).

6 Pick islets into new 35mm NTCC dish in **2 mL** of low glucose KRBH and pre-incubate in incubator at **37 °C** 5 % CO_2 for **01:00:00** . 1h

7 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 % CO_2. 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

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9 Gently add **500 µl** of low glucose KRBH to the islets, and incubate for **01:00:00** at **37 °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 °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 °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 °C** until insulin assay. **-20 °C**

15 Add **500 µl** of acid ethanol to the islets. Store tube at **-20 °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.

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