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Glucose-Stimulated Glucagon Secretion using Biorep Perifusion Machine - Mouse Islets

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1 *Works for me* dx.doi.org/10.17504/protocols.io.u6veze6

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MATERIALS TEXT

Sodium bicarbonate- Sigma Aldrich S5761
Bovine serum albumin, essentially fatty acid free- Sigma Aldrich A6003
Penicillin-Streptomycin- Gibco 15140122
RPMI 1640- Gibco 11875
FBS (Canadian origin)- Gibco 12483-020
Sodium Chloride- Fisher Scientific BP358-212
Potassium Chloride- Sigma Aldrich P9541
D Glucose- Sigma Aldrich G8270
Calcium Chloride- Sigma Aldrich C4901
Sodium Phosphate monobasic- Sigma Aldrich S5011
Magnesium Sulfate Heptahydrate- Sigma Aldrich M1880

Biorep perifusion machine
Deep well 96 well plates

MSD Mouse Glucagon ELISA: K150HCC-2
Alpco Rodent Insulin ELISA: 80-INSMR-CH01

Day before experiment

1 Isolate mouse islets as described in Mouse Islet Isolation protocol.

2 Pick the isolated mouse islets into Mouse Islet Culture Media (>90% islet purity).

Mouse Islet Culture Media

A	B
500 mL RPMI (11.1mMglucose)	Gibco 1875-119
50 mL FBS Canadian Origin	Gibco12483-020
5 mL Penicllin-Streptomycin	Gibco15140-122

3 Culture islets, up to 250 islets in 2 mL in a 35mm culture dish, overnight in incubator at 37°C, 5% CO2.

Solutions preparation that can be made in advance

4 Acid Ethanol for islet content collection

A	B
95% ethanol	150 mL
Acetic Acid	47 mL
Concentrated HCl	3 mL

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Solution preparation to be made fresh

5 KRBH solution to be made fresh:

A	B	C
	mM Final	per 100mL total volume
NaCl	140	7 mL (2M stock)
KCl	3.6	360 µL (1M stock)
CaCl2	2.6	260 µL (1M stock)
NaH2PO4	0.5	50 µL (1M stock)
MgSO4	0.5	50 µL (1M stock)
HEPES	5	500 µL (1M stock)
NaHCO3	2	0.0168 g
Essentially fatty acid free BSA	0.5mg/ml	50 mg

Warm above solution to 37°C (approximately 30min to1 hour). After warming the solution, pH to 7.45 with NaOH.

6 Add glucose and/or additional treatments as required.

Running the perifusion

7 Set up the chambers and perifusion machine according to Biorep instructions.

8 Set the protocol with glucose and experimental conditions (high KCl, inhibitors etc) and frequency of collection as needed using a flow rate of 100 µL/min for all steps.

Set step 1 of the perifusion protocol to total 30 min. The number of replicates will vary based on number of lanes and plate orientation. This is a preincubation step that should fill one 96 well plate. The perfusate from this step will be disgarded.

9 Prime the perifusion machine.

10 Load islets into each chamber. The number of islets will vary by experiment and sensitivity of ELISA.

11 Run a 30 min pre-incubation as previously set and discard the perfusate.

12 Continue to run the perifusion protocol as samples are collected into a clean deep well plate(s).

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13 Upon perifusion protocol is completion, run the chambers dry, so islets are on the filter paper but no liquid remains. Place the filter paper with islets into a 1.5 mL tube with 500 µL of acid ethanol.

14 Perfusate and islet content samples are stored at -20°C until ELISA

Glucagon ELISA

15 Perfusate is thawed and assayed using MSD glucagon Elisa kits. Vortex content sample and dilute 1:200

Insulin Elisa

16 If desired, samples are assayed for Insulin using Alpco Stellux mouse insulin ELISA. Vortex islet content and dilute 1:1000.

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