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Dynamic Glucose-Stimulated Insulin Secretion using Biorep Perifusion Machine (ver peri4.2) - Human or Mouse Islets

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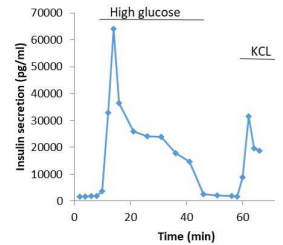
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Protocol status: Working

We use this protocol and it's working

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Abstract

General protocol for Dynamic Glucose-Stimulated Insulin Secretion using Biorep Perifusion Machine - Human or Mouse Islets.

Materials

Penicillin-Streptomycin- Gibco 15140122

RPMI 1640- Gibco 11875

DMEM- Gibco 11885

FBS (Canadian origin)- Gibco 12483-020

Sodium Chloride- Fisher Scientific BP358-212

Potassium Chloride- Sigma Aldrich P9541

Calcium Chloride- Sigma Aldrich C4901

Magnesium Chloride Hexahydrate- EMD millipore MX0045

HEPES- Fisher BP310-500

Sodium bicarbonate- Sigma Aldrich S5761

Bovine serum albumin- Sigma Aldrich A7906

D Glucose- Sigma Aldrich G8270

Biorep perifusion machine (ver peri4.2)

Deep well 96 well plates Fisher 12-565-606 or Greiner Bio-One 780280FD

Alpco stellux human Insulin ELISA: 80-INSHU-CH10

Alpco stellux Rodent Insulin ELISA: 80-INSMR-CH01



Day before experiment

1

For human islets:

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

	A	B
	500mL DMEM (5mM glucose)	Gibco 11885
	50 mL FBS Canadian Origin	Gibco 12483-020
	5 mL Penicillin- Streptomycin	Gibco 15140-122

2

For mouse islets:

Isolate mouse islets as described in [Mouse Islet Isolation](#) protocol.

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 Penicillin-Streptomycin	Gibco15140-122

3

For human and mouse islets:



Culture islets, up to 250 islets in 2 mL of media, in a 35mm non-treated 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

Solution preparation to be made fresh

5 KRBH solution to be made fresh on day of perfusion:

	A	B	C
		mM Final	per 100mL total volume
	NaCl	115	5.75 mL (2M stock)
	KCl	5	500 µL (1M stock)
	CaCl ₂	2.5	250 µL (1M stock)
	MgCl ₂	1	100 µL (1M stock)
	HEPES	10	1 mL (1M stock)
	NaHCO ₃	24	0.2g
	BSA	0.1% w/v	0.1g



Mix chemicals listed in the above table in milliQ water (approximately  80 mL).

Warm KRBH solution to  37 °C (approximately 30min to 1 hour). Once solution is warm, pH to 7.4 with NaOH and bring volume to  100 mL . KRBH should be kept at  37 °C for the duration of the experiment.

- 6 Add glucose and/or additional compounds as required.

Running the perfusion

- 7 Set up the chambers and perfusion 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 perfusion protocol to total 30 min. The number of replicates will vary based on number of lanes and plate orientation. This is a pre-incubation step that should fill one 96 well plate. The perfusate from this step will be discarded.
- 9 Prime the perfusion 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 (step 1 of perfusion machine protocol) and discard the perfusate.
- 12 Continue to run the perfusion protocol, collecting samples into a clean deep well plate(s).
- 13 Upon completion of perfusion protocol, seal the place deep well plates at store at  -20 °C until ELISA .



- 14 To collect insulin content, 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 μ L of acid ethanol. Store at  -20 °C until ELISA

Insulin Elisa

- 15 Samples are assayed for Insulin using Alpco Stellux human or mouse insulin ELISA. Vortex insulin content samples and dilute 1:400 prior to assay.