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**Protocol status:** Working  
We use this protocol and it's working

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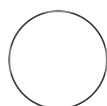
# Dynamic Glucose-Stimulated Insulin Secretion using Biorep Perifusion Machine (ver peri4.2) - Human or Mouse Islets

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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

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### 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 |

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### 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 |

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### 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% CO<sub>2</sub>.

## 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 $\mu$ L (1M stock) |
| CaCl <sub>2</sub>  | 2.5      | 250 $\mu$ L (1M stock) |
| MgCl <sub>2</sub>  | 1        | 100 $\mu$ L (1M stock) |
| HEPES              | 10       | 1 mL (1M stock)        |
| NaHCO <sub>3</sub> | 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  $\mu\text{L}/\text{min}$  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  $\mu$ 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.