Static glucagon secretion analysis of isolated islets

Julien Ghislain[1], Vincent Poitout[2], Caroline CT Tremblay[1], Marine Croze[1]

[1]Montreal Diabetes Research Center, CRCHUM, Montréal, QC, Canada;
[2]Montreal Diabetes Research Center, CRCHUM, and Department of Medicine, Université de Montréal, Montréal, QC, Canada

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Laboratory of Vincent Poitout
Tech. support email: julien.ghislain.chum@ssss.gouv.qc.ca

Julien Ghislain

This protocol describes the steps to measure glucagon secretion in a static, 1-hour assay from isolated pancreatic islets. It is suitable for islets isolated from both rodents and humans. We routinely apply this protocol to assess alpha-cell function in response to low glucose concentrations and L-arginine but can be easily adapted to interrogate the response to a variety of secretagogues (eg. fatty acids, hormones). Briefly batches of 10 islets are pre-incubated in triplicate in KRB solution at 5.5 mM glucose twice for 20 min followed by incubation in 1 mM glucose with or without 10 mM L-arginine for 1 hour. Secreted glucagon is measured in the supernatant and intracellular glucagon content, after acid-alcohol extraction, by radioimmunoassay. This protocol is also suitable for assessing SST secretion, however we recommend increasing the islet number per well from 10 to at least 20 due to the relative lower levels of SST compared to glucagon.

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protocol

Free fatty acid receptor 4 inhibitory signaling in delta cells regulates islet hormone secretion in mice. Croze ML, Flisher MF, Guillaume A, Tremblay C, Noguchi GM, Granziera S, Vivot K, Castillo VC, Campbell SA, Ghislain J, Huising MO, Poitout V. Mol Metab. 2021 Mar;45:101166. doi: 10.1016/j.molmet.2021.101166. PMID: 33484949

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Sodium Chloride **Fisher Scientific Catalog #S271**

Calcium Chloride Dihydrate **Fisher Scientific Catalog #C79**

Potassium Phosphate dibasic (KH2PO4) **Sigma Aldrich Catalog #P9791**

Potassium Chloride **Sigma – Aldrich Catalog #746436**

Magnesium sulfate heptahydrate (MgSO4) **Millipore Sigma Catalog #63138**

Sodium bicarbonate **Sigma Aldrich Catalog #S6014**

HEPES **Sigma Aldrich Catalog #H6147**

D-( )-Glucose **Sigma Aldrich Catalog #G-7528**

L-Arginine **Contributed by users Catalog #A5006**

Fatty Acid Free Heat Shock Bovine Serum Albumin Powder **equitech bio, inc. Catalog #BAH66-0500**

Ethanol (100%, Molecular Biology Grade) **Fisher Scientific Catalog #BP2818500**

RPMI 1640 Medium **Thermo Fisher Scientific Catalog #11875093**

FCS (Fetal Calf Serum) **Life Technologies**

Glucagon RIA **Sigma Aldrich Catalog #GL-32K**

Preparation of KRB solution and plates 20m

1 **Prepare KRB stocks, glucose and L-arginine solutions**

KRB stock I: weigh **27.7 g** NaCl bring to **1 L** with milliQ water

KRB stock II: weigh **1.494 g** $CaCl_2 \cdot 2H_2O$ and bring to **1 L** with milliQ water

KRB stock III: weigh **0.648 g** KH_2PO_4 and bring to **1 L** with milliQ water

KRB stock IV: weigh **1.415 g** KCl, **1.17 g** $MgSO_4 \cdot 7H_2O$ and **8.52 g** $NaHCO_3$ and bring to **1 L** with milliQ water

[M] **1 Molarity (M)** Glucose: weigh **18.02 g** and bring to **100 mL** with milliQ water. Filter sterilize using a 0.2 µm filter.

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1h

2 Prepare KRB solution

Determine the number of static conditions for the assay in order to prepare a sufficient volume of KRB. Remember that you will have two pre-incubation steps and the picking, along with extra media to wash between these steps. In a beaker combine equal volumes of the four KRB stock solutions to achieve the desired volume. Add [M]**2.38 mg/mL** HEPES powder and swirl to dissolve. Then add [M]**1 mg/mL** BSA (fatty acid free), but do not mix as the BSA will stick to the sides. Cover with plastic wrap (put holes in top) and place in the **37 °C** incubator for > **01:00:00** . Adjust the solution to pH **7.35** using [M]**1 Molarity (M)** NaOH. The solution should start at ~ pH **7.2** .

3 5.5 mM Glucose condition and islet picking and washing

Calculate the required volume of [M]**5.5 millimolar (mM)** Glucose in KRB for the two islet pre-incubations (**1 mL** /well) plus **1 mL** /well and about **70 mL** for the islet picking and wash steps. Add **5.5 µL** of [M]**1 Molarity (M)** Glucose/ml of KRB.

1 mM Glucose condition

Calculate the required volume of [M]**1 millimolar (mM)** Glucose in KRB for the static (**1 mL** /well) and add **1 µL** of [M]**1 Molarity (M)** Glucose/ml of KRB needed.

1 mM Glucose plus 10 mM L-arginine condition

Calculate the required volume of [M]**1 millimolar (mM)** Glucose and [M]**10 millimolar (mM)** L-arginine in KRB for the static (**1 mL** /well) and add **1 µL** of [M]**1 Molarity (M)** Glucose/ml of KRB . Then add L-arginine in powder form ([M]**1.75 mg/mL**) and adjust to pH **7.35** with HCl.

> Often there are other reagents to be added to the final static conditions, such as fatty acids, inhibitors or agonists. These additional components may require that separate KRB solutions be prepared. In the case of fatty acids addition prepare the KRB solution without BSA.

4 Prepare plates

Prepare islet picking plates by adding **1 mL** of [M]**5.5 millimolar (mM)** Glucose in KRB to three wells of a 24-well plate for each static sample.

Prepare pre-incubation plates by adding **1 mL** of [M]**5.5 millimolar (mM)** Glucose in KRB to three wells of a 24-well plate for each static sample. Repeat this step for a second pre-incubation plate. Place these plates in an incubator with [M]**5 % volume** CO2 at **37 °C** .

Prepare static incubation plate by adding **1 mL** of experimental KRB ([M]**1 millimolar (mM)** glucose alone or [M]**1 millimolar (mM)** glucose plus [M]**10 millimolar (mM)** L-arginine) to three wells of a 24-well plate for each static sample. Place these plates in the incubator with [M]**5 % volume** CO2 at **37 °C** .

Islet Picking and Incubations 2h 45m

2h 50m

Following isolation, the islets should be allowed to recover in recovery medium (RPMI / 10% serum /

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5 [M]**11.1 millimolar (mM)** glucose) for **01:00:00** at **37 °C** . Wash the islets in a petri dish containing **20 mL** of [M]**5.5 millimolar (mM)** Glucose in KRB.
Pick the islets (in triplicate batches of 10) into the islet picking plate wells.
Using a pipette transfer the islets from the picking plate to the first pre-incubation plate. Incubate at **37 °C** for **00:20:00** .

Then transfer the islets to the second pre-incubation plate. Incubate at **37 °C** for **00:20:00** .

Then transfer the islets to the incubation plate and incubate at **37 °C** for **01:00:00** .

During the incubation, label two **1.5 mL** tubes per sample for collection of the KRB media containing secreted glucagon. Prepare and label **1.5 mL** tubes filled with **1 mL** acidified ethanol ([M]**75 % (v/v)** ethanol / [M]**1.5 % (v/v)** HCl) for glucagon content analysis.
At the end of the static incubation, collect the islets and transfer them to the tubes pre-filled with acidified ethanol. Cap the vials and store at **-20 °C** overnight.

Then, transfer the media from each well of the static incubation into a **1.5 mL** tube, centrifuge at **4000 rpm, 4°C, 00:05:00** , transfer the supernatant to a new **1.5 mL** tube and store at **-20 °C** until ready to complete the glucagon assay.
The next day retrieve the glucagon content analysis tubes, vortex and centrifuge at **4000 rpm, 4°C, 00:05:00** . Transfer the supernatant to labelled **1.5 mL** tubes. Store the glucagon content samples at **-20 °C** , until ready to complete the glucagon assay.

Radioimmunoassay

6 Radioimmunoassay kits are used to measure glucagon levels. Several kits are available from MilliporeSigma. For the protocol please refer to the manufacturer's instruction.

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