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Static insulin secretion analysis of isolated islets

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We use this protocol and it's working

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Abstract

This protocol describes the steps to measure insulin 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 beta-cell function in response to glucose 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 2.8 mM glucose twice for 20 min followed by incubation in either 2.8 mM or 16.7 mM glucose for 1 hour. Secreted insulin is measured in the supernatant and intracellular insulin 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 insulin.

Materials

- Sodium Chloride **Fisher Scientific** **Catalog #**S271
- Calcium Chloride Dihydrate **Fisher Scientific** **Catalog #**C79
- Potassium Phosphate dibasic (KH2PO4) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**P9791
- Potassium Chloride **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**746436
- Magnesium sulfate heptahydrate (MgSO4) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**63138
- Sodium bicarbonate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S6014
- HEPES **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**H6147
- D-( )-Glucose **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**G-7528
- 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**
- Rat insulin RIA **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**RI-13K
- Human Insulin-Specific RIA **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**HI-14K

Troubleshooting

Preparation of KRB solution and plates

1 **Prepare KRB stocks**

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.

2 **Prepare KRB solution** 1h

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 **2.8 mM Glucose condition and islet picking and washing**

Calculate the required volume of [M] 2.8 millimolar (mM) Glucose in KRB for the two islet pre-incubations and static (1 mL /well) plus 1 mL /well and about 70 mL for the islet picking and wash steps. Add 2.8 µL of [M] 1 Molarity (M) Glucose/ml of KRB.

16.7 mM Glucose condition

Calculate the required volume of [M] 16.7 millimolar (mM) Glucose in KRB for the static (1 mL /well) and add 16.7 µL of [M] 1 Molarity (M) Glucose/ml of KRB needed.

Note

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] 2.8 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] 2.8 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 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 40m

5 2h 50m

Following isolation, the islets should be allowed to recover in recovery medium (RPMI / [M] 10 % (v/v) serum / [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] 2.8 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 insulin. 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 insulin 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 insulin assay.

The next day retrieve the insulin 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 insulin content samples at -20 °C , until ready to complete the insulin assay.

Radioimmunoassay

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