Assessment of Islet Functional Potency by Glucose Stimulated Insulin Secretion

PURPOSE: To assess the in vitro functional potency of purified islets by measuring the amount of insulin release upon glucose stimulation.

SPECIMEN: Purified human Islet preparation.

APPLICABLE FORMS AND ATTACHMENTS:
Glucose Stimulated Insulin Secretion (GSIS) Assay Worksheet

MATERIALS:

Preparation of reagents:

Stock Kreb's Buffer (KRB)

Chemical	F.W.	[mM]	g/L
NaCl	58.44	137	8.0
KCl	74.55	4.7	0.44
KH_2PO_4	136.1	1.2	0.16
$MgSO_4$-$7H_2O$	246.48	1.2	0.3
$CaCl_2$-$2H_2O$	147	2.5	0.37
$NaHCO_3$	84.01	25	2.1

Working Kreb's buffers (3.3 mM glucose, 16.7 mM glucose)

- Bring 100 ml of stock KRB to 37°C by heating in a water bath.
- Oxygen saturate 100 ml of stock KRB with 95% O_2/5% CO_2 10 – 15 minutes by continuous bubbling.
- Add 0.05 g of BSA, ELISA grade, mix to ensure complete suspension.
- pH to 7.4 with HCL or NaOH if necessary.
- Remove 25 ml and place in a 50 ml conical tube.
- Add 150 µl of sterile 1.65 M Glucose D (+) solution to the 75 ml of oxygen saturated KRB – BSA solution to make the **3.3 mM glucose Basal working solution**.
- Add 250 µl of sterile 1.65 M Glucose D (+) solution to the 25 ml of oxygen saturated KRB – BSA solution to make the **16.7 mM glucose working solution**.
- Use solutions immediately.

EQUIPMENT:

- Shaking waterbath set at 37°C
- Micrometer syringe connected to silicone tubing and a sterile Pasteur pipet.
- Inverted microscope.
- Microfuge tubes (0.5 or 1.5 ml) labeled (Isolation code, sample code, date)
- Cryovials (1.5 ml) labeled (Isolation code, sample code, date)
- Petri dishes, p100 or p60

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PROCEDURE

1. Obtain approximately 150 islet particles from a designated flask from the Quality Control tissue culture incubator.
2. Pipet islets into a p100 Petri dish containing sufficient pre-warmed culture media to wet the entire dish (~20 ml).
3. Prefill two Petri dishes with 25 ml of Basal buffer (3.3 mM glucose KRB). Label "Basal #1" and "Basal #2".
4. Pipet 0.5 ml of Basal buffer into 5 cryovials labeled (3.3-1, 3.3-2, 3.3-3, 3.3-4, and 3.3-5).
5. Pipet 0.5 ml of 16.7 mM glucose KRB into 5 cryovials labeled (16.7-1, 16.7-2, 16.7-3, 16.7-4, and 16.7-5).
6. Place the media filled cryovials into a 37^{o}C tissue culture incubator.
7. Place the Petri dish containing the islets on the inverted microscope.
8. Using a Pasteur pipet connected to a micrometer syringe, careful pick ~150 islet particles and place in the Basal #1 Petri dish.
9. Place the Basal #1 dish containing islets and the Basal #2 dish into the 37^{o}C tissue culture incubator and incubate for 30 minutes.
10. Place the Basal #1 Petri dish containing the islets on the inverted microscope.
11. Using a Pasteur pipet connected to the micrometer syringe, careful pick the islet particles from the Basal#1 Petri dish and place into the Basal #2 Petri dish.
12. Place the Basal #2 Petri dish containing the islets on the inverted microscope.
13. Carefully handpick 8-10 islets using the Pasteur pipet and transfer to each of the 10 cryovials. Place the screw caps on the cryovials without tightening. *Be sure to minimize the total volume of Basal media transferred.*
14. Incubate the islets in a 37°C waterbath for 60 minutes, with gentle shaking.
15. Pellet the islets by touch spin in a microcentrifuge.
16. Carefully remove 0.3 ml supernatant and dispense into the labeled microfuge tubes.
17. Save the tubes containing the islets either for immediate DNA extraction or store at -80^{o}C.

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18. Insulin is measured by ELISA from the supernatant sample. Attach ELISA worksheets containing raw OD data and extrapolated insulin values to the GSIS Assay Worksheet. Recommended product is the *Linco Research "Human Insulin ELISA Kit" Cat. No. EAHI-14K*.

19. DNA is extracted from the islets remaining in the cryovials and quantified by standard methods. Attach DNA quantification worksheets/printouts containing raw absorbance or fluorescence values and extrapolated DNA concentrations to the GSIS Assay Worksheet. Recommended product is the *Qiagen DNAeasy kit*.

DATA RECORDING AND ANALYSIS

1. Insulin and DNA values are recorded on the GSIS Assay Worksheet in the "Raw Data" section and in a new Excel worksheet file within the UW Islet Program Quality Control Database.
2. Each insulin value is normalized to the DNA content extracted from the islets in the exact cryovial from which the insulin was quantified and recorded in the "Originals" section of the data sheet..
3. The highest and lowest DNA normalized insulin values are flagged to minimize variance.
4. The non-flagged DNA normalized insulin data are recorded in the "Processed" section of the Assay Worksheet.
5. The Stimulation Index (S.I.) is calculated by dividing the Mean DNA normalized insulin values measured from the 16.7 mM glucose samples by the 3.3 mM glucose samples. S.I.'s for the Original and Processed data are recorded on the Assay Worksheet.

CLEAN UP PROCEDURE:

- Place all disposable items in a biohazard waste container, and sharps in biohazard sharp container.

SAFETY:

- Always wear gloves, protective eyewear, and observe UNIVERSAL PRECAUTIONS.

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- Dispose of contaminated disposable items in Biohazard Waste. Disinfect reusable items properly before washing.

REFERENCES:

- Marchetti P, Scharp DW, McLear M, Gingerich R, Finke E, Olack B, Swanson C, Giannarelli R, Navalesi R, Lacy PE: Pulsatile insulin secretion from isolated human pancreatic islets, Diabetes, 1994, June; 43 (6):827-30.

Glucose Stimulated Insulin Secretion Assay Worksheet

Isolation Code _____ **Isolation Date** _____ **GSIS Assay Date**_____

GSIR Assay Performed by: _____ **ELISA Performed by:** _____ **DNA Quantification performed by:** _____

RAW DATA

	Insulin [µU/ml]	DNA [µg/ml]
[Glucose]	Originals	Originals
3.3-1		
3.3-2		
3.3-3		
3.3-4		
3.3-5		
16.7-1		
16.7-2		
16.7-3		
16.7-4		
16.7-5		
Mean Low		
Mean High		

DNA NORMALIZED DATA

	Insulin [µU/mL]/DNA [µg/mL]		Insulin [µU/mL]/DNA [µg/mL]
[Glucose]	Originals	Flagged High/Low [√]	Processed
3.3-1			
3.3-2			
3.3-3			
3.3-4			
3.3-5			
16.7-1			
16.7-2			
16.7-3			
16.7-4			
16.7-5			
Mean Low			
SD Low			
Mean High			
SD High			
Stimulation Index (SI)			

Reviewed by: _____ **Date:** _____

Approved by: _____ **Date:** _____

Matthew S. Hanson, Ph.D., Associate Director, Islet Core Facility