

Document Title:

**PURIFIED HUMAN PANCREATIC ISLETS,
GLUCOSE STIMULATED INSULIN RELEASE DETERMINATION BY ELISA,
ATTACHMENT III, SOLUTIONS PREPARATIONS**

1. Krebs Buffer Stock Solution Preparation

Follow instructions in SOP 3104, A03

Material	Source	FW	Lot #	Exp. Date	Quantity Required			Quantity Used
					1 Liter	0.5 Liter	0.25 Liter	
HEPES powder		238.3			5.955 g	2.978 g	1.489 g	g
NaCl		58.44			6.721 g	3.361 g	1.680 g	g
NaHCO ₃		84.01			2.012 g	1.006 g	0.5029 g	g
KCl		74.55			0.3728 g	0.1864 g	0.0932 g	g
MgCl ₂ 6 H ₂ O		203.3			0.2033 g	0.1017 g	0.0508 g	g
CaCl ₂ .2 H ₂ O		147.0			0.3675 g	0.1838 g	0.0919 g	g
BSA					1.000 g	0.5000 g	0.2500 g	g
Deionized Water					q.s. to 1 L	q.s. to 0.5 L	q.s. to 0.25 L	L

- Check pH of the solution and adjust to 7.3 to 7.5 using either 1 N NaOH or 1 N HCl, if necessary.
- Filter sterilize into a sterile container using a 0.22 µm sterile filter.
- If the formula weight (FW) does not match that listed, recalculate the quantity required in the solution according to the final concentration listed in SOP 3104, A03, page 4 of 11, V. A1.

Prepared by: _____ **Date:** _____

2. Stock 280 mM Glucose Solution Preparation

Follow instructions in SOP 3104, A03

Material	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
D-(+)-Glucose				2.5 g	g
Krebs Buffer Stock Solution				50 mL	mL

The amount listed can be adjusted proportionately depending on what is needed. Sterile the solution to a 50 mL conical tube by using a 0.22 µm syringe filter.

Prepared by: _____ **Date:** _____

Pre-culture Islets Lot #: _____

Islets Product Lot #: _____

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3. High Glucose (28 mM) Solution Preparation

Follow instructions in SOP 3104, A03

Material	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
Stock 280 mM Glucose Solution				5 mL	mL
Krebs Buffer Stock Solution				45 mL	mL

The amount listed can be adjusted proportionately depending on what is needed.

Prepared by: _____ **Date:** _____

4. Low Glucose (2.8 mM) Solution Preparation

Follow instructions in SOP 3104, A03

Material	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
High Glucose (28 mM) Solution				5 mL	mL
Krebs Buffer Stock Solution				45 mL	mL

The amount listed can be adjusted proportionately depending on what is needed.

Prepared by: _____ **Date:** _____

5. High Insulin Control Preparation

Follow instructions in SOP 3104, A03

Material	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
High Insulin Control				1 vial	vial
Deionized Water				500 µL	µL

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6. Low Insulin Control Preparation

Follow instructions in SOP 3104, A03

Material	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
Low Insulin Control				1 vial	vial
Deionized Water				500 µL	µL

Prepared by: _____ **Date:** _____

7. Wash Solution Preparation

Follow instructions in SOP 3104, A03

Material	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
Wash Buffer 21X				40 mL	mL
Deionized Water				800 mL	mL

Prepared by: _____ **Date:** _____

8. Enzyme Conjugate Solution Preparation

Follow instructions in SOP 3104, A03

Material	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
Enzyme Conjugate 11X				100 µL	µL
Enzyme Conjugate Buffer				1 mL	mL

Prepared by: _____ **Date:** _____

Reviewed by: _____ **Date:** _____