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**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

Marriel Samuel	175X/	7557 F -4-44	Exp.	Quantity Required			Quantity	
Material	Source	FW	Lot #	Date	1 Liter	0.5 Liter	0.25 Liter	Used
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 <sub>3</sub>		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 <sub>2</sub> 6 H <sub>2</sub> O		203.3			0.2033 g	0.1017 g	0.0508 g	g
CaCl <sub>2</sub> .2 H <sub>2</sub> 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

Prepared b	oy:	Date:			
	t listed can be adjust tube by using a 0.22	ted proportionately dep 2 µm syringe filter.	ending on what i	s needed. Sterile t	he solution to a 50
Solution				50 mL	mL
				ΓΛT	T

Pre-culture Islets Lot #:	_ Islets Product Lot #:	

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_	ucose (28 mM) Soluti	•			
Follow i	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
Stock 280 mM Glucose Solution	1			5 mL	mL
Krebs Buffer Stock Solution				45 mL	mL
	ount listed can be adju	sted proportionately	depending on what is	s needed.	<u> </u>
Prepar	ed by:	Date	<b>:</b> :		
4. Low Glu	ucose (2.8 mM) Solut	ion Preparation			
Follow i	Source	Lot #	Expiration Date	Quantity Required	<b>Quantity Used</b>
High Glucose (2 mM) Solution	8		Dute	5 mL	mL
Krebs Buffer Stock Solution				45 mL	mL
The amo			depending on what is	s needed.	
_	ed by:		:		
	sulin Control Preparat				
Material	Source	Lot #	Expiration Date	Quantity Required	<b>Quantity Used</b>
High Insulin Control				1 vial	vial
Deionized Water	ſ			500 μL	μL
Prepare	ed by:	Date	:		
Pre-culture Islet	s Lot #•		Islets Product Lo	ot #•	

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	PURIF	IED HUMAN PA	ANCREATIC ISL	ETS,	
GLUCO	SE STIMULATE	D INSULIN REI	LEASE DETERM	INATION BY	ELISA,
	ATTACHM	IENT III, SOLU	JTIONS PREPAR	RATIONS	
6. Low Ins	ulin Control Preparation	on			
Follow i	nstructions in SOP 31	04, A03			
Material	Source	Lot #	Expiration Date	Quantity Required	<b>Quantity Used</b>
Low Insulin Control				1 vial	vial
Deionized Water	•			500 μL	μL
Prepare	ed by:	Date	:		
7. Wash So	olution Preparation				
Follow i	nstructions in SOP 31	04, A03			
Material	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
Wash Buffer 212	X			40 mL	mL
Deionized Water				800 mL	mL
Prepare	ed by:	Date	:		
8. Enzyme	Conjugate Solution Pr	reparation			
Follow i	nstructions in SOP 31	04, A03			
Material	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
Enzyme Conjugate 11X				100 μL	μL
Enzyme Conjugate Buffe	r			1 mL	mL
Prepared by: Date:					
•	ed by:				
Reviewo	eu by:	Date	<b>:</b>		

Pre-culture Islets Lot #:	Islets Product Lot #: