Purified Human Pancreatic Islets CIT Enzyme Solution Roche Enzymes – *Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium*

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Document Title:

PURIFIED HUMAN PANCREATIC ISLETS
CIT ENZYME SOLUTION
ROCHE ENZYMES

Manufacturing Site: _____ Date: _____

1.0 Materials:

Material	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
Hanks' Balanced Salt Solution (HBSS)				500 or 1000 mL	mL
Heparin Sodium Injection USP, Preservative free		_____Units/mL		_____mL	mL

Note: Add 10u/ml of heparin in HBSS

1.1a

Material	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
Calcium Chloride USP (Dihydrate) ($CaCl_2$ $2H_2O$)				160 or 320 mg	mg
1M HEPES (To dissolve $CaCl_2$ $2H_2O$)				12.8 or 25.6 mL	mL

OR

1.1b

Material	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
Calcium Chloride Injection USP ($CaCl_2$), 10% Solution				1.6 or 3.2 mL	mL

Islets Lot Number: _____

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1.2 Enzyme solution

Material	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
HBSS with Heparin				q.s. to 350 to 500 mL	mL
Sterile Water For Injection USP					
Liberase MTF C/T GMP Grade - Collagenase	Roche			1 or 2 Containers	Container(s)
Liberase MTF C/T GMP Grade - Thermolysin	Roche			1 or 2 Containers	Container(s)

2.0 Procedure

2.1 Preparation of HBSS and Calcium Chloride solution

2.1.1 Prepare 500 or 1000 mL of HBSS with Heparin concentration of 10 U/ mL.

HBSS Volume: _____ mL Heparin Volume: _____ mL

2.1.2 When using powder, add 160 mg (or 320 mg) of $CaCl_2$ $2H_2O$ to a 50 mL conical tube.

2.1.3 Add 12.8 mL (or 25.6 mL) of 1M HEPES to the 50 mL conical tube and mix to dissolve.

2.1.4 Filter the Calcium Chloride solution through a 0.22 micron filter.

OR

When using Calcium Chloride Injection USP, 10% solution (13.6 meq/10 mL), add 1.6 mL (or 3.2 mL) at the time of enzyme preparation.

2.2 Determine the weight of pancreas: Calculate the Estimated Final Trimmed Pancreas Weight (C) based on the Initial Trimmed Pancreas Weight (A) from PBR Section 5.7, and estimated cannulae, fat, blood vessel and connective tissue weight (B): A – B = C

Initial Trimmed Pancreas Weight (g) (A)	Estimated cannulae, fat, blood vessel and connective tissue weight (g) (B)	Estimated Final Trimmed Pancreas Weight (g) (C)

2.3 Determine the volume of enzyme to be prepared:

Estimated Final Trimmed Pancreas Weight (g) (C)	CIT Enzyme Solution Final Volume (mL)
<100	350
100-125	400
126-150	450
>151*	500

* For a pancreas >150 g, there is an option to divide the pancreas into two portions and digest these separately

Final volume of enzyme solution: _____mL

Islets Lot Number: _____

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2.4 Determine a target collagenase quantity to be used according to characteristics of the donor and pancreas, and record the rationale in the comments section below. For most donor pancreata, 21 – 32 Wünsch units per gram Estimated Final Trimmed Pancreas Weight is desirable. For older donors or more fibrotic organs, this value may be increased by up to 50% at the discretion of the manufacturing team lead.

Target Collagenase Concentration (D): _____ Wünsch Units/g pancreas

Comments: _____

_____

2.4.1 Calculate the amount of collagenase needed (E): C X D = E

Estimated Final Trimmed Pancreas Weight (g) (C)	Target Collagenase Concentration (Wünsch Units/g) (D)	Collagenase needed (Wünsch Units) (E)

2.4.2 In a BSC about 45 minutes before the start of perfusion, aseptically add 20 – 40 mL HBSS (prepared in Section 2.1 above) to each vial (1 or 2 based on Wünsch units calculated in Section 2.2.3 above) of Collagenase. Maintain at cold temperature (2 to 8ºC recommended) until completely dissolved. Occasionally, swirl gently and avoid creating air bubbles.

2.4.3 Calculate the volume of Collagenase solution to use in order to have the Wünsch Units of Collagenase needed:

20 – 40 mL/vial X Collagenase Units needed = X mL of Collagenase solution to use
Wünsch Units/vial

_____ mL/vial X _____ (E) Units = _____ mL of Collagenase solution to use

_____ Wünsch Units/vial

2.5 Transfer the dissolved enzyme to a sterile bottle containing 300 mL of cold (2 to 8ºC recommended) HBSS (prepared in Section 2.1 above).

2.6 Determine a target Thermolysin concentration according to characteristics of the donor and pancreas, and record your rationale in the comments section below. For most donor pancreata, 600 to 2000 units per gram Estimated Final Trimmed Pancreas Weight is desirable. For younger donors, this value may be increased by up to 50% at the discretion of the manufacturing team lead. For ischemically damaged pancreata, this value may be decreased by up to 50% at the discretion of the manufacturing team lead.

Target thermolysin concentration (F): _____units/g pancreas

Comments: _____

_____

Islets Lot Number: _____

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2.7 Calculate the amount of Thermolysin needed (G): C X F = G

Estimated Final Trimmed Pancreas Weight (g) (C)	Target Thermolysin Concentration (Units/g) (F)	Thermolysin needed (Units) (G)

2.8 Aseptically add 5-10 mL of Sterile Water for Injection USP to Thermolysin bottle (each 1or 2 bottles) based on units needed (G), calculated in Section 2.6.2 above) vial of Thermolysin. Maintain at cold (2 to 8°C recommended) temperature until completely dissolved. Occasionally, swirl gently and avoid creating air bubbles.

2.9 Calculate the volume of Thermolysin solution to use in order to have the units of Thermolysin oe Neutral Protease needed (G):

5 – 10mL/vial X Thermolysin Units needed / Units/vial = X mL Thermolysin solution to use
OR
Neutral Protease

_____mL/vial X _____Units= _____ mL Thermolysin or Neutral Protease solution to use

_____Units/vial

2.10 Add the Thermolysin (or Neutral Protease)solution to the bottle containing the Collagenase solution immediately before use and swirl gently to mix. Avoid creating air bubbles.

2.11 Label the bottle with:

- "CIT Enzyme Solution"
- "Volume prepared _____ mL"
- "Store at 2°C to 8°C"
- Date and Time Prepared (*mmddyyyy, 24 hour clock*)
- Expiration Date and Time (one half hour after preparation) (*mmddyyyy, 24 hour clock*)
- Initials of the person who prepared the solution

2.12 After final pancreas trimming, determine the final enzyme units used per g of trimmed pancreas:

Actual (not Estimated) Final Trimmed Pancreas Weight (PBR Section 6.3) (H): _____ g

2.12.1 Collagenase (Wünsch Units) / Final Trimmed Pancreas Weight (g): E/H = J

Collagenase Used (Wünsch Units) (E)	Final Trimmed Pancreas Weight (g) (H)	Collagenase Units/g (Wünsch Units/g) (J)

2.12.2 Thermolysin (Units) / Final Trimmed Pancreas Weight (g): G/H = K

Thermolysin Used (Units) (G)	Final Trimmed Pancreas Weight (g) (H)	Thermolysin Units/g (Units/g) (K)

Islets Lot Number: _____

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2.13 Based on the final enzyme calculations, the timing and temperature of pancreas digestion should be adjusted to maximize islet yield. For example, if the final enzyme units used per g of trimmed pancreas (J or K) exceeds the target (D or F), the temperature set-point during digestion or the length of pancreas digestion can be lowered to compensate. When large differences from the target exist (poor estimation of final trimmed pancreas weight), the digest should be carefully monitored to adjust these parameters and determine the optimal switch point. Briefly describe the rationale for any adjustments made to the timing or temperature of pancreas digestion.

Comments: _____

_____

_____

_____

Prepared by: _____ **Date:** _____

Reviewed by: _____ **Date:** _____

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