

Purified Human Pancreatic Islets: CIT Enzyme Solution Vitacyte Enzymes and Vitacyte/SERVA Enzymes Combination – *Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium*

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SOP ATTACHMENT



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Document Title:

PURIFIED HUMAN PANCREATIC ISLETS CIT ENZYME SOLUTION VITACYTE ENZYMES AND VITACYTE/SERVA ENZYMES COMBINATION

Manufacturing Site: _____ Date: _____

1.0 Materials:

Material	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
Hanks' Balanced Salt Solution (HBSS)				q.s. to 350 to 500 mL	mL
Sterile Water for Injection USP				10 mL	mL
Heparin Sodium Injection USP, Preservative Free		_____ Units/mL		10 Units/mL Enzyme Sol.	Units mL
CIzyme Collagenase HA	VitaCyte			1 or 2 Containers	Container(s)
Select one of the two below:					
CIzyme Thermolysin	VitaCyte			1 or 2 Containers	Container(s)
Neutral Protease NB <input type="checkbox"/> Premium Grade <input type="checkbox"/> GMP Grade	SERVA			1 – 5 Containers	Container(s)

2.0 Procedure

2.1 Determine the number of units of VitaCyte Collagenase required by using the following steps:

2.1.1 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)

Islets Lot Number: _____

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2.1.2 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, 20-24 Wunsch units per gram trimmed pancreas is desirable. For younger 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): _____ Wunsch Units/g pancreas

Comments: _____

2.1.3 Calculate the amount of collagenase needed (E): $C \times D = E$

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

2.2 In a BSC about 45 minutes before the start of perfusion, aseptically add 20 mL HBSS to each vial (1 or 2 based on Wunsch units calculated in Section 2.1, above) of VitaCyte Collagenase. Maintain at cold temperature (2 to 8°C recommended) until completely dissolved. Occasionally swirl gently. Avoid creating air bubbles.

Start time: _____ End time: _____ Reconstitution time: _____ minutes

Start time: _____ End time: _____ Reconstitution time: _____ minutes

2.3 Calculate the volume of Vitacyte Collagenase solution to use in order to have the Wunsch Units of Collagenase needed:

$$\frac{20 \text{ mL/vial} \times \text{Collagenase Units needed}}{\text{Wunsch Units/vial}} = \text{mL of Vitacyte Collagenase solution to use}$$

$$\frac{20 \text{ mL/vial} \times \text{ (E) Units}}{\text{Wunsch Units/vial}} = \text{_____ mL of Vitacyte Collagenase solution to use}$$

2.4 Transfer the dissolved enzyme to a sterile 500 mL bottle containing 300 mL of cold (2 to 8°C recommended) HBSS.

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- 2.5 Calculate the volume of Heparin Sodium Injection USP solution required to achieve 10 U heparin/mL Final Enzyme Solution (see section 2.8 below for Final Enzyme Solution Volume) with the equation below and add it to the 500 mL bottle.

$$\frac{10 \text{ U Heparin/mL Enzyme Solution} \times \text{mL Final Enzyme Solution}}{\text{Units of Heparin/mL}} = \text{mL Heparin Solution required}$$

$$\frac{10 \text{ U/mL} \times \text{mL Enzyme Solution}}{\text{Units of Heparin/mL}} = \text{mL Heparin Solution required}$$

- 2.6 Determine the number of units of VitaCyte Thermolysin or SERVA Neutral Protease needed by using the following steps:

- 2.6.1 Determine a target protease concentration according to characteristics of the donor and pancreas, and record your rationale in the comments section below. For most donor pancreata, 1.5 to 2.5 DMCU or 0.01 to 0.035 MFU per gram Estimated Final Trimmed Pancreas Weight is desirable. For younger donors or more fibrotic organs, this value may be increased by up to 50% at the discretion of the manufacturing team lead.

Target protease concentration (F): _____ DMCU or MFU (circle one)/g pancreas

Comments: _____

- 2.6.2 Calculate the amount of protease needed (G): $C \times F = G$

Estimated Final Trimmed Pancreas Weight (g) (C)	Target Protease Concentration (DMC or MF Units/g) (F)	Protease needed (DMC or MF Units) (G)

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

Start time: _____ End time: _____ Reconstitution time: _____ minutes

Start time: _____ End time: _____ Reconstitution time: _____ minutes

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- 2.8 Calculate the volume of Vitacyte Thermolysin or SERVA Neutral Protease solution to use in order to have the units of Protease needed (G):

$\frac{10 \text{ mL/vial} \times \text{DMC or MF Units}}{\text{DMC or MF Units/vial}} = \text{mL Thermolysin or Neutral Protease solution to use}$

$\frac{10 \text{ mL/vial} \times \text{Units}}{\text{Units/vial}} = \text{mL Thermolysin or Neutral Protease solution to use}$

- 2.9 Total enzyme volume may be selected based on pancreas size, ranging from 350 to 500 mL. Q.S. the 500ml bottle to 350 to 500 ml minus the volume of Thermolysin or Neutral Protease calculated above (Section 2.8). Final enzyme volume should be determined based on the table below:

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

- 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:
- Either
“CIT Enzyme Solution – VitaCyte Collagenase and VitaCyte Thermolysin”
 - Or (Cross out the unused identification)
“CIT Enzyme Solution – VitaCyte Collagenase and Serva Neutral Protease”
 - “Volume prepared _____ mL”
 - “Store at 2°C to 8°C”
 - Date and Time Prepared (*mmdyyyy*, 24 hour clock)
 - Expiration Date and Time (one half hour after preparation) (*mmdyyyy*, 24 hour clock)
 - Initials of the person who prepared the solution

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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 Protease (DMC or MF Units) / Final Trimmed Pancreas Weight (g): G/H = K

Protease Used (DMC or MF Units) (G)	Final Trimmed Pancreas Weight (g) (H)	Protease Units/g (DMC or MF Units/g) (K)

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 setpoint 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:** _____

Islets Lot Number: _____