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Purified Human Pancreatic Islets, CIT Enzyme Solution-SERVA Enzymes Proportional Units Collagenase & Neutral Protease – Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium

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Division of Allergy, Immunology and Transplantation

Standard Operating Procedure (Attachment)



National Institute of Allergy and Infectious Diseases

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Document Title:
**Purified Human Pancreatic Islets,
CIT Enzyme Solution-SERVA Enzymes
Proportional Units Collagenase & Neutral Protease**

Manufacturing Site: _____ Date: _____

1.0 Hanks' Balanced Salt Solution, 1X + 10 U/mL Heparin

1.1 Materials:

Material	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
Hanks' Balanced Salt Solution, 1X				1 L	L
Heparin Sodium Injection USP, Preservative Free		_____ Units/mL		_____ mL (See below)	mL

1.2 Procedure

Prepare 1 liter of HBSS, 1X with 10 U/mL of heparin. Calculate the amount of heparin to be added to the liter of HBSS, 1X to produce 10 U/mL heparin.

$$\frac{10 \text{ U/mL in media} \times 1,000 \text{ mL}}{\text{U/mL in heparin solution}} = \text{mL heparin solution to use}$$

$$\frac{10 \text{ U/mL in media} \times 1,000 \text{ mL}}{\text{U/mL heparin}} = \text{_____ mL}$$

Calculated by: _____ Date: _____

Verified by: _____ Date: _____

Islets Lot Number: _____

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2.0 Enzyme Solution

2.1 Materials:

Material	Source	Lot #	Expiration Date	Quantity Required	Quantity Used
Hanks' Balanced Salt Solution, 1X + 10 U/mL heparin		See Section 1.1 above		~330 to ~480 mL	mL
Collagenase NB 1 <input type="checkbox"/> GMP Grade <input type="checkbox"/> Premium Grade	SERVA			Calculated below	mL
Calcium Chloride Injection USP (CaCl ₂), 10% Solution				5.2 mL	mL
1 M HEPES				35 mL	mL
Neutral Protease NB <input type="checkbox"/> GMP Grade <input type="checkbox"/> Premium Grade	SERVA			Calculated below	mL
Sterile Water for Injection				10 mL	mL

* Calcium Chloride and HEPES may or may not be used; determined by islet processing lead.

Based on the Cannulated Pancreas Weight (MPBR Section 5.8) and the table below, determine the volume of this solution to make and the amount of enzymes to use.

Cannulated Pancreas Weight (MPBR Section 5.8): _____ g

Choose Total Enzyme Solution Volume: 350 mL, or 400 mL, or 450 mL, or 500 mL (circle one)

Example: Suggested Calculations for a 110 gram pancreas.

Note: Any suggested range combination of Collagenase can be combined with any suggested range of Neutral Protease; final enzyme concentration and combination to be determined by islet processing lead.

Choose one of the following:

Collagenase Units to Use = Pancreas Weight X Target Collagenase Units/g pancreas

Pancreas Weight (g)	Enzyme Target Concentration can be:	Suggested Collagenase (Units/g)	Suggested Collagenase Used (Units)
110	Low	16-19	1760-2090
	Medium Low	20-24	2200-2640
	Medium	25-27	2750-2970
	Medium High	28-31	3080-3410
	High	32	3520

Islets Lot Number: _____

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Choose one of the following:

Neutral Protease Units to Use = Target Collagenase Units/ g pancreas

Pancreas Weight (g)	Enzyme Target Concentration can be:	Suggested Neutral Protease (Units/g)	Suggested Neutral Protease Used (Units)
110	Low	1.00-1.70	110-187
	Medium Low	1.80-2.50	198-275
	Medium	2.60-3.40	286-374
	Medium High	3.50-4.40	385-484
	High	4.50	495

Worksheet:

ENTER Pancreas Weight (g)	Enzyme Solution Volume (mL):	Collagenase Used (Units)	Collagenase (Units/g Panc.)	Neutral Protease Used (Units)	Neutral Protease (Units/g Panc.)

Final Collagenase concentration: _____ Wunsch Units/mL

Final Neutral Protease concentration: _____ DMC Units/mL

2.2 Collagenase NB 1 Reconstitution

2.2.1 **ABOUT 45 MINUTES BEFORE PERFUSION** add 40 mL of HBSS, 1X + heparin to one vial of Collagenase NB 1.

2.2.2 Let collagenase dissolve at 2°C – 8°C (about 30 minutes). Swirl gently occasionally.

	Start Time	End Time	Reconstitution Time (min)
Collagenase Vial #1			
Collagenase Vial #2			

2.3 Label a sterile 500 mL 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*)

Islets Lot Number: _____

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- Initials of the person who prepared the solution
- 2.4 To the 500 mL bottle; add ½ the chosen volume of Hanks’ Balanced Salt Solution, 1X + 10 U/mL heparin.
- 2.5 *Optional step: To the 500 mL bottle; add Calcium Chloride and/or HEPES.
- 2.6 Calculate volume of Collagenase NB-1 solution to use in order to have the chosen (Section 2.1, above) number of units:

$$\frac{40 \text{ mL/vial} \times \text{Collagenase Units chosen}}{\text{Stock Wünsch Units/vial}} = \text{mL Collagenase NB-1 to use}$$

$$\frac{40 \text{ mL/vial} \times \text{_____}}{\text{Stock Wünsch Units/vial}} = \text{_____ mL}$$

- 2.7 Add the calculated volume of dissolved Collagenase NB-1 to the sterile 500 mL bottle.
- 2.8 Calculate the volume of Neutral Protease NB solution to use in order to have the determined (Section 2.1, above) number of units:

$$\frac{10 \text{ mL/vial} \times \text{Neutral Protease Units chosen}}{\text{Stock DMC-Units/vial}} = \text{mL Neutral Protease to use}$$

$$\frac{10 \text{ mL/vial} \times \text{_____}}{\text{Stock DMC-Units/vial}} = \text{_____ mL}$$

- 2.9 Neutral Protease NB Reconstitution
 Add 10 mL of Sterile Water for Injection to one vial of Neutral Protease and mix to dissolve.

	Start Time	End Time	Reconstitution Time (min)
Neutral Protease Vial #1			
Neutral Protease Vial #2			
Neutral Protease Vial #3			

- 2.10 Q.S the 500 mL bottle to the volume chosen in Section 2.1, above, minus the volume of Neutral Protease NB solution to be added, with HBSS, 1X + heparin. Swirl gently to mix.
- 2.11 Add the calculated volume of dissolved Neutral Protease NB to the sterile 500 mL bottle **immediately** before the start of perfusion.

Prepared by: _____ Date: _____

Reviewed by: _____ Date: _____

Islets Lot Number: _____