

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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**SOP
ATTACHMENT**



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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				~330 to ~480 mL	mL
Collagenase NB 1 <input type="checkbox"/> GMP Grade <input type="checkbox"/> Premium Grade	SERVA			Calculated below	mL
Calcium Chloride USP (Dihydrate) (CaCl ₂ 2 H ₂ O)				0.52 g	g
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

2.1 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 (g)	CIT ENZYME SOLUTION VOLUME (mL)	COLLAGENASE (UNITS)	NEUTRAL PROTEASE (UNITS)
< 100	350	1600	200
100 – 125	400	1829	229
126 – 150	450	2057	257
> 150*	500	2286	286

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

Cannulated Pancreas Weight (MPBR Section 5.8): _____ g

CIT Enzyme Solution Volume to be prepared: _____ mL

Collagenase: _____ Units Neutral Protease: _____ Units

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.

2.3 Calcium Chloride 11 mM

2.3.1 Add 0.52 g Calcium Chloride USP (dihydrate) to a 50 mL sterile conical tube.

2.3.2 Add 35 mL of 1 M HEPES to the 50 mL conical tube and mix to dissolve.

2.3.3 Filter the CaCl₂ solution through a 0.22 micron filter into a sterile 500 mL bottle.

Islets Lot Number: _____

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2.4 Calculate volume of Collagenase NB-1 solution to use in order to have the determined (Section 2.1, above) number of units:

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

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

2.5 Add the calculated volume of dissolved Collagenase NB-1 to the sterile 500 mL bottle.

2.6 Calculate the volume of Neutral Protease NB solution to use in order to have the determined (Section 2.1, above) number of units:

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

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

2.7 Neutral Protease NB Reconstitution

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

2.8 Q.S the 500 mL bottle to the volume determined in Section 2.1, above, minus the volume of Neutral Protease NB solution to be added, with HBSS, 1X + heparin. Swirl gently to mix.

2.9 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

2.10 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: _____