Purified Human Pancreatic Islets, CIT Purification Density Gradients – *A Standard Operating Procedure of the NIH Clinical Islet Transplantation Consortium*

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Document Title:

PURIFIED HUMAN PANCREATIC ISLETS, CIT PURIFICATION DENSITY GRADIENTS

Manufacturing Site: _____ Date: _____

1. Materials:

Material	Source	For #1, Preparation Date For #2 & #3, Lot #	Expiration Date
1. CIT Purification Solution	Made In-house		
2. OptiPrep	Nycomed, Product #1114542		
3. Gradient Stock Solution	Mediatech, Product No. 99-674-CM		

2. Procedure

2.1 **High Purification Density Gradient, 1.10 g/mL** ± 0.01 g/mL

Material	Quantity Required	Initial Quantity Used	Additional Quantity Used
CIT Purification Solution	81.25 g	g	g
Gradient Stock Solution	468.75 g	g	g

2.1.1 In a BSC place a sterile 500 mL bottle on a balance.
2.1.2 Add 81.25 g of CIT Purification Solution to the bottle.
2.1.3 Add 468.75 g of Gradient Stock Solution to the bottle.
2.1.4 Cap the bottle and mix by gentle inversion at least five times.
2.1.5 Check that the weight is 550.0 g. Observed Weight: _____ g
2.1.6 Check the density of the solution with a densitometer.
Measured Density: _____ g/mL
If the density is not 1.09 g/mL to 1.11 g/mL, the solution's density may be adjusted by adding the higher or lower density component. Record the weight of component added in the table, above. If the density cannot be adjusted into the range, the solution may not be used. Initiate and document an investigation of the process and materials.

Islets Lot Number: _____

CellR4 2015; 2 (3): e991 – www.cellr4.org – ISSN: 2329-7042

2.1.7 Label the bottle with:
- "High Purification Density Gradient, 1.10 g/mL"
- Islets Lot Number
- "Store at 2°C to 8°C"
- Date Prepared
- Expiration Date (the end of the day after preparation)
- Initials of the person who prepared the gradient

2.1.8 Store the bottle of solution at 2°C to 8°C before use.

Total volume prepared: _____ mL

Prepared by: _____ **Date:** _____

Reviewed by: _____ **Date:** _____

2.2 **Low Purification Density Gradient 1.06 g/mL** ± 0.01 g/mL

Material	Quantity Required	Initial Quantity Used	Additional Quantity Used
CIT Purification Solution	245.7 g	g	g
OptiPrep	29.9 g	g	g

2.2.1 In a BSC place a sterile 500 mL bottle on a balance.
2.2.2 Add 245.7 g of CIT Purification Solution to the bottle.
2.2.3 Add 29.9 g of OptiPrep to the bottle.
2.2.4 Cap the bottle and mix by gentle inversion at least five times.
2.2.5 Check that the weight is 275.6 g. Observed Weight: _____ g
2.2.6 Check the density of the solution with a densitometer.
Measured Density: _____ g/mL
If the density is not 1.05 g/mL to 1.07 g/mL, the solution's density may be adjusted by adding the higher or lower density component. Record the weight of component added in the table, above. If the density cannot be adjusted into the range, the solution may not be used. Initiate and document an investigation of the process and materials.
2.2.7 Label the bottle with:
- "Low Purification Density Gradient, 1.06 g/mL"
- Islets Lot Number
- "Store at 2°C to 8°C"
- Date Prepared
- Expiration Date (the end of the day after preparation)
- Initials of the person who prepared the gradient

2.2.8 Store the bottle of solution at 2°C to 8°C before use.

Total volume prepared: _____ mL

Prepared by: _____ **Date:** _____

Reviewed by: _____ **Date:** _____

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