



Version 2

Apr 30, 2021

# Thawing Cryopreserved Human Islets V.2

In 1 collection

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Works for me

[dx.doi.org/10.17504/protocols.io.bt6dnra6](https://dx.doi.org/10.17504/protocols.io.bt6dnra6)James Lyon  
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## ABSTRACT

This protocol details the thawing of human islets following their cryopreservation, as performed by the Alberta Diabetes Institute IsletCore. The cryopreservation method is detailed in [Human Islet Cryopreservation](#) protocol. Human islets cryopreserved and thawed using these methods have been found to retain viability and function after 20 years of cryogenic biobanking.

Manning Fox JE, Lyon J, Dai XQ, Wright RC, Hayward J, van de Bunt M, Kin T, Shapiro AMJ, McCarthy MI, Gloyn AL, Ungrin MD, Lakey JR, Kneteman NM, Warnock GL, Korbitt GS, Rajotte RV, MacDonald PE (2015) Human islet function following 20 years of cryogenic biobanking. [Diabetologia](#), 58(7): 1503-1512.

## DOI

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## PROTOCOL CITATION

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Version created by Jocelyn E Manning Fox

## COLLECTIONS ⓘ

**ADI IsletCore Protocols for the Isolation, Assessment and Cryopreservation of Human Pancreatic Islets of Langerhans for Research Purposes**

## WHAT'S NEW

Minor edits for clarity.

## LICENSE

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## PROTOCOL INTEGER ID

49061

## PARENT PROTOCOLS

Part of collection

ADI IsletCore Protocols for the Isolation, Assessment and Cryopreservation of Human Pancreatic Islets of Langerhans for Research Purposes

## MATERIALS TEXT

### MATERIALS

[HEPES](#) **Fisher**

**Scientific Catalog #BP310-500**

[EMD Millipore™ Stericup™ Sterile Vacuum Filter Units](#) **Fisher**

**Scientific Catalog #SCGPU05RE**

[Medium 199](#)

**powder Corning Catalog #90-050-PC**

[Sodium Bicarbonate](#) **Fisher**

**Scientific Catalog #S233**

[Penicillin/streptomycin](#) **Lonza Catalog #09-757F**

[CMRL 1066](#)

**medium Corning Catalog #15-110-CV**

[Glutamax \(100x\)](#) **Gibco - Thermo**

**Fischer Catalog #35050-061**

[Insulin Transferrin Selenium](#)

**(20x) Corning Catalog #25-800-CR**

[Bovine Serum Albumin](#) **equitech bio,**

**inc. Catalog #BAL62-1000**

[sucrose](#) **Fisher**

**Scientific Catalog #BP220-1**

[HyClone Fetal Bovine Serum](#) **Fisher**

**Scientific Catalog #SH3039603**

[Sterile Cellulose Acetate Syringe Filters](#) **Fisher**

**Scientific Catalog #09-302-156**

## BEFORE STARTING

Ensure solutions are prepared.

M199 solution

1

A	B	C	D	E
<b>M199 (10L)</b>				
<b>Reagent</b>	<b>Concentration</b>	<b>Mass/Volume</b>	<b>Supplier</b>	<b>Catalogue #</b>
M199 powder	9.41g/L	1 bottle	Mediatech-Corning	90050PB 3
NaHCO <sub>3</sub>	26 mM	22.0g	Fisher Scientific	S233-500
HEPES	10 mM	23.83g	Fisher Scientific	BP310-1
Penicillin/Streptomycin	20,000 U/ml penicillin and 20,000 µg/ml streptomycin	50ml	Lonza	09-757F

Prepare the M199 solution using the above Media Preparation table above:

1. Dispense **9 L** of Milli-Q (18mΩ) water into a carboy
2. Store overnight at **4 °C** to allow to come to temperature.
3. Using a stirrer add the M199 media powder to the water and allow to mix into solution.
4. Add the powdered supplements and Penicillin/Streptomycin to the appropriate media based on the above table and stir into solution.
5. Stir the solution for **00:30:00**
6. Store the prepared solution overnight at **4 °C** to allow all powders to go into the solution
7. Stir the solution for **00:30:00**
8. Calibrate the pH meter using the pH control buffers
9. Adjust the pH level of the M199 solution to **pH7.4** using the NaOH and/or HCl.
10. Bring to the volume of 10L with the appropriate amount of Milli-Q water (18mΩ).  
Filter into 1L bottles with a 0.22µm nitrocellulose filter.

## Sucrose Solution

### 2

Heat inactivation of FBS

1. Thaw serum and aliquot into labelled 50ml tubes. *If serum was thawed in a refrigerator allow serum to come to room temperature prior to placing in water bath.*
2. Fill the water-bath with sufficient water so that the tubes may be submersed to the level of the serum.
3. Set water-bath temperature to maintain the product at **56 °C**.
4. Once **56 °C** is reached, place the tubes in the water-bath for **00:30:00**.
5. After 30 minutes immediately remove the tubes from the water bath.
6. Store the tubes of heat inactivated FBS at **-20 °C**.

### 3 Freeze M199:

Prepare Freeze M199 solution as follows:

Add **100 mL** heat inactivated FBS (HyClone™ Fetal Bovine Serum (Canada), Characterized - Fisher cat# SH3039603) to **900 mL** M199.

Store at **4 °C** . Warm to room temperature for use.

#### 4 Prepare 0.75M sucrose in freeze M199 and place on ice.

1. Weigh **12.836 g** sucrose and transfer to a 50 ml beaker.
2. Add **30 mL** of freeze M199 (as above) and mix until in solution
3. Transfer sucrose solution to a 50 ml volumetric flask, add freeze M199 to bring total volume to 50 ml.
4. Filter sterilize with a 0.22 um cellulose acetate filter.

### Human Islet Culture media

#### 5



















A	B	C	D	E	F
Reagent	CMRL 1066	Bovine serum albumin 30%	Insulin-Transferrin-Selenium (100x)	Gibco® GlutaMAX Supplement	Penicillin-Streptomycin Mixture
Volume	500 ml	8.5 ml	5 ml	10 ml	2.5 ml

CMRL 1066 - Corning Catalogue number [15110CV](#)  
Bovine serum albumin 30% v/v - Equitech Bio Inc. Catalogue number [BAL62](#)  
Insulin-Transferrin-Selenium (100x) - Corning Catalogue number [25800CR](#)  
Gibco® GlutaMAX™ Supplement (100x) - ThermoFisher catalogue number [35050061](#)  
Penicillin-Streptomycin Mixture - Lomza Catalogue number [09-757F](#)

Prepare Human Islet Culture media as described in the table above. Filter sterilize using a Stericup Sterile Vacuum Filtration System.

### Thawing protocol

- 6 Remove tubes of cryopreserved human islets from Liquid Nitrogen dewar and place into portable bath containing liquid nitrogen.
- 7 Immerse and agitate tubes in **37 °C** water bath until almost thawed (WARNING: do not over thaw, remove the tube from the water bath when a small piece of ice is still present).
- 8 Transfer to **0 °C** ice slush
- 9 Centrifuge tubes at **282 x g, 4°C, 00:01:00**
- 10 Remove supernatant
- 11 Add **1 mL** of cold 0.75M sucrose. Lightly hand vortex to get pellet back into suspension.  
Hold on **0 °C** ice slush for **00:30:00** .

- 12 Transfer the samples to  **Room temperature** for  **00:01:00** .
- 13 Continue at  **Room temperature** . Add  **1 mL** of freeze M199 and lighty mix. Incubate for  **00:05:00** .
- 14 Add  **1 mL** of freeze M199 and lighty mix. Incubate for  **00:05:00** .  
 **Room temperature** .
- 15 Add  **2 mL** of freeze M199 and lighty mix. Incubate for  **00:05:00** .  
 **Room temperature** .
- 16 Add  **4 mL** of freeze M199 and lighty mix. Incubate for  **00:05:00**  
 **Room temperature** .
- 17 Centrifuge the sample tubes at  **282 x g, Room temperature , 00:01:00**
- 18 Remove supernatant and add  **5 mL** of Human Islet Culture media and lightly hand vortex to allow the islets to go into suspension.  
 **Room temperature** .
- 19 Using Human Islet Culture media, culture islets at  **22 °C** 5% CO<sub>2</sub> or use for experiments. It is recommended to hand pick the islets to purity as soon as possible after thawing.