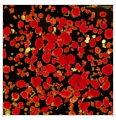

Thawing Cryopreserved Human Islets

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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 crypreservation 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 20years 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, Korbutt GS, Rajotte RV, MacDonald PE (2015) Human islet function following 20 years of cryogenic biobanking. Diabetologia, 58(7): 1503-1512.

PROTOCOL STATUS

Working

We use this protocol in our group and it is working

MATERIALS

NAME	CATALOG #	VENDOR
HEPES	BP310-500	Fisher Scientific
EMD Millipore™ Stericup™ Sterile Vacuum Filter Units	SCGPU05RE	Fisher Scientific
Medium 199 powder	90-050-PC	Corning
Sodium Bicarbonate	S233	Fisher Scientific
Penicillin/streptomycin	09-757F	Lonza
CMRL 1066 medium	15-110-CV	Corning
Glutamax (100x)	35050-061	Gibco - Thermo Fischer
Insulin Transferrin Selenium (20x)	25-800-CR	Corning
Bovine Serum Albumin	BAL62-1000	equitech bio, inc.
sucrose	BP220-1	Fisher Scientific
HyClone Fetal Bovine Serum	SH3039603	Fisher Scientific
Sterile Cellulose Acetate Syringe Filters	09-302-156	Fisher Scientific

BEFORE STARTING

Ensure solutions are prepared.

M199 solution

1

M199 (10L)			Supplier	Supplier Catalogue #	Link
M199 powder	9.41g/L	1 bottles (1x10L/bottle)	Mediatech-Corning	90050PB 3	
NaHCO3	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 9L of Milli-Q (18mΩ) water in to 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 table (step 1) and allow to stir into solution.
5. Stir the solution for 30 min
6. Store the prepared solution overnight at 4°C to allow all powders to go into solution
7. Stir the solution for 30 min
8. Calibrate the pH meter using the buffer controls
9. Adjust the pH level of M199 solution to 7.4 using the NaOH and/or HCl.
10. Bring to volume of 10L with the appropriate amount of Milli-Q water (18mΩ).
11. Filter into 1L bottles with a 0.22µm nitrocellulose filter.

Sucrose Solution

2 **Freeze M199:**

Prepare Freeze M199 solution as follows:

Add 100ml heat inactivated FBS (HyClone™ Fetal Bovine Serum (Canada), Characterized - Fisher cat# SH3039603) to 900ml M199 Store at 4oC. Warm to room temperature for use.

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 30 minutes.
5. After 30 minutes immediately remove the tubes from the water bath.
6. Store the tubes of heat inactivated FBS @ -20°C.

3 Prepare 0.75M sucrose 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

4

Media	**Total /bottle (ml)**	**BSA (ml)**	**CMRL 1066 (ml)**	**ITS (ml)**	**Glutamax (ml)**	**Pen/strep (ml)**
Culture Media	**521**	**8.5 (0.5%)**	**500**	**5**	**5**	**2.5**
		Equitech Bio Inc.	Mediatech/Corn	Mediatech/Corn	Gibco	Lonza
		BL64	15110CV	25800CR	35050061	09-757F

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

Thawing protocol -Step 1

5 Remove tubes of cryo preserved human islets from Liquid Nitrogen dewar and place into portable bath containing liquid nitrogen.

Thawing protocol - Step 2

6 Immerse and agitate tubes in 37ºC water bath until almost thawed (WARNING: do not over thaw the cells, remove tube from waterbath when last small piece of ice is still present).

37 °C

Thawing protocol - Step 3

7 Transfer to 4ºC ice slush.

4 °C **Ice slush**

Thawing protocol - Step 4

8 Centrifuge tubes at 282 x g for 1 min at 4ºC

00:01:00

4 °C **centrifuge 1min @ 282 x g**

Thawing protocol - Step 5

9 Remove supernatant

Thawing protocol - Step 6

10 Add 1mL of cold 0.75M sucrose. Lightly hand vortex to get pellet back into suspension.
Hold on ice slush for 30 min.

1 ml **0.75M sucrose**

00:29:00

4 °C

Thawing protocol - Step 7

11 Transfer the samples to room temperature for 1 minute.

00:01:00

22 °C

Thawing protocol - Step 8

12 Add 1mL of freeze M199 and incubate for 5 min.

00:05:00

1 ml **freeze M199**

22 °C

Thawing protocol - Step 9

13 Add 1mL of freeze M199 and incubate for 5 min.

00:05:00

1 ml freeze M199

22 °C

Thawing protocol - Step 10

14 Add 2mL of freeze M199 and incubate for 5 min.

00:05:00

2 ml freeze M199

22 °C

Thawing protocol - Step 11

15 Add 4mL of freeze M199 and incubate for 5 min.

00:05:00

4 ml freeze M199

22 °C

Thawing protocol - Step 12

16 Centrifuge the sample tubes at 282 x g for 1min at Room temperature.

22 °C

00:01:00

Thawing protocol - Step 13

17 Remove supernatant and add 5ml of Human Islet Culture media and lightly hand vortex to allow the islets to go into suspension.

22 °C culture

Thawing protocol - Step 14

18 Using Human Islet Culture media, culture islets at 22°C - 5% CO_2 or use for experiments. It is recomended to hand pick the islets to purity as soon as possible after thawing.