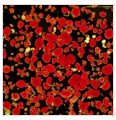

Human Islet Cryopreservation

James Lyon[1], Patrick MacDonald[1], Jocelyn Manning Fox[1]
[1]University of Alberta

dx.doi.org/10.17504/protocols.io.spcediw

Jocelyn Manning Fox
University of Alberta

ABSTRACT

This protocol details the cryopreservation of human islets, as performed by the Alberta Diabetes Institute IsletCore. Human islets cryopreserved using this method 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
Cryopreservation storage tubes (12ml)	3775	Thermo Scientific
DMSO	BP231	Fisher Scientific
Sodium bicarbonate	S233-500	Fisher Scientific
HEPES	BP310-1	Fisher Scientific
Penicillin/Streptomycin	09-757F	Lonza
M199 medium	MT90050PB	Fisher Scientific
Brady FREEZERBONDZ Polyester thermal transfer printer labels	22-500521	Fisher Scientific
HyClone Fetal Bovine Serum	SH3039603	Fisher Scientific
Syringe Filters Nylon 0.2µm pore Sterile	N7262520	Fisher Scientific
FTS Multi Cool low temperature bath	MC880	LabWrench

BEFORE STARTING

Ensure solutions are prepared and cryopreservation bath is precooled.

Preparation of Solutions

1

M199 Media Preparation Table

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.

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 ar 4°C. Warm to room temperature prior to 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.

2M DMSO:

- Add enough Freeze M199 to 7.8g of DMSO (Fisher Scientific - Cat# BP231)for a total volume of 50ml.
- Filter sterilize through a syringe filter (Nylon 0.2µm pore) into a sterile glass media bottle
- Store ar 4°C. Warm to room temperature prior to use.

3M DMSO:

- Add enough Freeze M199 to 11.7g of DMSO (Fisher Scientific - Cat# BP231)for a total volume of 50ml.
- Filter sterilize through a syringe filter (Nylon 0.2µm pore) into a sterile glass media bottle.
- Store ar 4°C. Warm to room temperature prior to use.

Pre-cool cryopreservation bath

2 Pre-cool FTS bath and set temperature at –7.4°C starting temp approximately 2 hours prior beginning the cryopreservation.

Label the cryopreservation tubes

3 Label cryopreservation tubes using Brady™ FREEZERBONDZ™ Polyester Thermal Transfer Printer Labels (Fisher Scientific Cat #22-500521). Include the following information.

- Internal Identifier number (Rxxx)
- Number of islet equivalents (IEQ) per tube
- Cryoprotectant (DMSO)
- Cryopreservation date
- Tube number

Protocol - Step 1

4 Aliquot islets into the cryopreservation tubes with a maxiumum of 25,000 IEQ per cryopreservation tube.

Protocol - Step 2

5 1. Centrifuge all tubes @ 280xg for 1 minute @4°C and remove the supernatant.

01:00:00

4 °C

Protocol - Step 3

6 Add 1ml of freeze M199 to each tube and suspend the islets. (RT)

1 ml **Freeze M199**

22 °C

Protocol - Step 4

7 Add 500µl 2M DMSO and start timer. (RT)

500 µl **2M DMSO**

22 °C 00:00:00

Protocol - Step 5

8 At 5 minutes add an additional 500µl 2M DMSO - (RT)

500 µl **2M DMSO**

22 °C

Protocol - Step 6

9 At 30 minutes add 2ml 3M DMSO - (RT)

2 ml **3M DMSO**

22 °C

Protocol - Step 7

10 At 45 minutes transfer all tubes to a rack in an ice slush.(4°C)

4 °C ice bath

Protocol - Step 9

11 At 55 minutes transfer all tubes to the pre-chilled FTS bath (-7.5°C)

-7.5 °C Seeding bath

Protocol - Step 10

12 At 65 minutes nucleate* all tubes to release the latent heat of fusion

-7.5 °C Nucleation

*Nucleation is necessary to remove the latent heat of fusion. At this point the tissue suspension is "super-cooled". This refers to the fact that the suspension is liquid despite being at -7.5°C. Nucleation is achieved by submersing each tube for 2 seconds in liquid nitrogen and then applying a mechanical force (firmly hit the tube against a solid object). If nucleation is successful the solution will appear to be a "slush". Immediately after nucleation return each tube back to the seeding bath.

Protocol - Step 8

13 At 85 minutes begin the FTS bath rate cooling procedure at -2.5°C/min

-7.5 °C cool down by 2.5°C/min

Protocol - Step 11

14 Once the FTS bath reaches -40°C all tubes can be transferred to liquid nitrogen

-40 °C

Storage

15 Once the cryopreservation protocol is complete and the tubes have been transferred to liquid nitrogen (-174.5°C), the tubes can be transferred to long term storage in a liquid nitrogen vapour phase. This vapour phase can be achieved by using a Chart MVE 1500 Series LN_2 storage tank or similar system.

-196 °C store in LN2 vapour phase

Human islets cryopreserved in this manner can be successfully thawed using the Thawing Cryopreserved Human Islets protocol.