Version 2

Apr 30, 2021

Human Islet Cryopreservation Version 2.0 V.2

In 1 collection

James Lyon[1], Aliya F Spigelman[1], Jocelyn E E Manning Fox[1], Patrick E Macdonald[1]

[1]University of Alberta

1 Works for me dx.doi.org/10.17504/protocols.io.bt6cnraw

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

DOI

dx.doi.org/10.17504/protocols.io.bt6cnraw

PROTOCOL CITATION

James Lyon, Aliya F Spigelman, Jocelyn E E Manning Fox, Patrick E Macdonald 2021. Human Islet Cryopreservation Version 2.0. **protocols.io**
https://dx.doi.org/10.17504/protocols.io.bt6cnraw
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 clarification.

CREATED

Apr 12, 2021

LAST MODIFIED

Apr 30, 2021

PROTOCOL INTEGER ID

49060

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

Cryopreservation storage tubes (12ml) **Thermo Scientific Catalog #3775**

DMSO **Fisher Scientific Catalog #BP231**

Sodium bicarbonate **Fisher Scientific Catalog #S233-500**

HEPES **Fisher Scientific Catalog #BP310-1**

Penicillin/Streptomycin **Lonza Catalog #09-757F**

M199 medium **Fisher Scientific Catalog #MT90050PB**

Brady FREEZERBONDZ Polyester thermal transfer printer labels **Fisher Scientific Catalog #22-500521**

HyClone Fetal Bovine Serum **Fisher Scientific Catalog #SH3039603**

Syringe Filters Nylon 0.2µm pore Sterile **Fisher Scientific Catalog #N7262520**

FTS Multi Cool low temperature bath **LabWrench Catalog #MC880**

BEFORE STARTING

Ensure solutions are prepared and cryopreservation bath is precooled.

Preparation of Solutions

1

M199 Media Preparation Table

A	B	C	D	E
M199 (10L)	**Concentration**	**Weight/Volume**	**Supplier**	**Catalogue #**
M199 powder	9.41g/L	1 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 **9 L** 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 above table and allow to stir into solution.
5. Stir the solution for **00:30:00**

Citation: James Lyon, Aliya F Spigelman, Jocelyn E E Manning Fox, Patrick E Macdonald (04/30/2021). Human Islet Cryopreservation Version 2.0. https://dx.doi.org/10.17504/protocols.io.bt6cnraw

6. Store the prepared solution overnight at **4 °C** to allow all powders to go into 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 M199 solution to **pH 7.4** using the NaOH and/or HCl.
10. Bring to volume of **10 L** with the appropriate amount of Milli-Q water (18mΩ).

Filter into **1 L** bottles with a 0.22µm nitrocellulose filter.

2 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 media.

Store at **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 **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 2M DMSO:

- Add Freeze M199 to **7.8 g 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 at **4 °C** .
- Warm to room temperature prior to use.

4 3M DMSO:

- Add Freeze M199 to **11.7 g DMSO** 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 at **4 °C** .

Citation: James Lyon, Aliya F Spigelman, Jocelyn E E Manning Fox, Patrick E Macdonald (04/30/2021). Human Islet Cryopreservation Version 2.0. https://dx.doi.org/10.17504/protocols.io.bt6cnraw

- Warm to room temperature prior to use.

Pre-cool cryopreservation bath

5 Pre-cool FTS bath and set temperature at **-7.4 °C** starting temp approximately **02:00:00** prior beginning the cryopreservation.

Label the cryopreservation tubes

6 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

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

8 Centrifuge all tubes **280 x g, 4°C, 00:01:00** . After centrifugation, remove the supernatant. 1m

9 At room temperature, add **1 mL** of freeze M199 to each tube and suspend the islets.
22 °C

10 At room temperature, add **500 µl** 2M DMSO and start timer.
22 °C **00:00:00**

11 At 5 minutes add an additional **500 µl** 2M DMSO.
22 °C

12 At 30 minutes add **2 mL** 3M DMSO.
22 °C

Citation: James Lyon, Aliya F Spigelman, Jocelyn E E Manning Fox, Patrick E Macdonald (04/30/2021). Human Islet Cryopreservation Version 2.0. https://dx.doi.org/10.17504/protocols.io.bt6cnraw

13 At 45 minutes transfer all tubes to a rack in an ice bath.

0 °C ice bath

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

-7.5 °C Seeding bath

15 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.

16 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

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

Storage

18 Once the cryopreservation protocol is complete and the tubes have been transferred to liquid nitrogen (-190°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.

-190 °C store in LN2 vapour phase

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

Citation: James Lyon, Aliya F Spigelman, Jocelyn E E Manning Fox, Patrick E Macdonald (04/30/2021). Human Islet Cryopreservation Version 2.0. https://dx.doi.org/10.17504/protocols.io.bt6cnraw