

Contents

1. Description

1.1 Background information

1.2 Applications

1.3 Reagent requirements

2. Protocol

2.1 Freezing of human iPS cells passaged as single cells

2.2 Freezing of human iPS cells passaged as cell clusters

2.3 Thawing of human iPS cells

2.4 Freezing of human iPS-derived cardiomyocytes

1. Description

This product is for research use only.

Components	50 mL StemMACS Cryo-Brew
Specifications	pH: 7.4–7.8
Quality control	Functionality assay: Satisfactory replating efficiency of cryopreserved human pluripotent stem cells after thawing. Low endotoxin level by Limulus Amoebocyte Lysate (LAL) assay.
Storage	Store the StemMACS Cryo-Brew, human protected from light at 2–8 °C. The expiration date is indicated on the vial label.

1.1 Background information

StemMACS Cryo-Brew is an animal component-free medium formulation designed for xeno- and serum-free cryopreservation of human pluripotent stem cells (PSCs) and mesenchymal stem cells (MSCs). Cells frozen in StemMACS Cryo-Brew show high viability and rapid recovery after thawing.

1.2 Applications

Cryopreservation of human:

- PSCs,
- PSC-derived cells, for example, cardiomyocytes or dopaminergic progenitors,
- mesenchymal stem cells.

1.3 Reagent requirements

- Buffer: Dubecco's phosphate-buffered saline (DPBS) without Ca²⁺ and Mg²⁺.

Additional requirements for freezing of human iPS cells passaged as single cells

- 0.05% Trypsin/EDTA (alternatively, Accutase® or TrypLE™) and Soybean Trypsin Inhibitor (0.5 mg/mL) for single cell splitting.

Additional requirements for freezing of human iPS cells passaged as cell clusters

- StemMACS Passaging Solution XF (# 130-104-688) for passaging in cell clusters.

Additional requirements for thawing of human iPSC

- A small molecule ROCK inhibitor, e.g., StemMACS Y27632 (# 130-103-922) or StemMACS Thiazovivin (# 130-104-461) to improve cell attachment and survival.

Additional requirements for freezing of human iPSC-derived cardiomyocytes

- Multi Tissue Dissociation Kit 3 (# 130-110-204)

2. Protocol

2.1 Freezing of human iPS cells passaged as single cells

1. Culture cells in a 6-well plate until they reach 60% to 80% confluency.
2. Aspirate supernatant and wash each well with 3 mL of buffer.
3. Add 0.7 mL of 0.05% Trypsin/EDTA per well (alternatively, use Accutase® or TrypLE™). Gently rock the plate to ensure distribution of the enzyme solution.
4. Incubate for 5 minutes at 37 °C.
5. Stop enzymatic reaction by adding 2 mL of Soybean Trypsin Inhibitor (0.5 mg/mL) per well.
6. Use a 5 mL serological pipette to dissociate to a single-cell suspension by carefully pipetting up and down.
7. Determine cell number.
8. Transfer desired cell number into a 15 mL conical tube. Calculate with 10⁶ cells per 1 mL aliquot.
9. Centrifuge for 5 minutes at 200×g.
10. Aspirate supernatant.
11. Resuspend the cell pellet in StemMACS Cryo-Brew to 10⁶ cells per mL.
12. Quickly transfer the cell suspension into cryogenic vials (1 mL per vial).
13. Place the vials into an isopropanol freezing container and immediately store at –80 °C.
14. After 24 hours transfer cells into a liquid nitrogen tank for long-term storage.

2.2 Freezing of human iPS cells passaged as cell clusters

1. Culture cells in a 6-well plate until they reach 60% to 80% confluency.
2. Aspirate supernatant and wash each well with 3 mL of buffer.
3. Add 1 mL of StemMACS Passaging Solution XF per well. Gently rock the plate to distribute the solution evenly.
4. Incubate at room temperature for 4 minutes. Monitor the detachment process under the microscope.
5. Carefully remove the StemMACS Passaging Solution XF.
6. Add 2 mL of StemMACS Cryo-Brew to each well.
7. Detach the colonies by carefully pipetting up and down using a 5 mL serological pipette.
8. Quickly transfer the cell suspension into cryogenic vials (1 mL per vial).
9. Place the vials into an isopropanol freezing container and immediately store at -80°C .
10. After 24 hours transfer cells into a liquid nitrogen tank for long-term storage.

2.3 Thawing of human iPS cells

▲ Work quickly to avoid loss of cells.

1. Take a vial with cells out of the liquid nitrogen container.
2. Incubate the vial in a 37°C water bath until only a little lump of ice is left.
3. Quickly transfer cell suspension into a 15 mL conical tube and dropwise add 5 mL of used cell culture medium.
4. Centrifuge for 5 minutes at $200\times g$.
5. Aspirate supernatant.
6. Resuspend the cell pellet in the culture medium supplemented with a small molecule ROCK inhibitor.
7. Seed 70,000–150,000 cells per well ($7000\text{--}16,000$ cells/ cm^2) in appropriately coated 6-well plates.

2.4 Freezing of human iPSC-derived cardiomyocytes

1. Harvest cells using the Multi Tissue Dissociation Kit 3.
2. (Optional) Isolate differentiated cardiomyocytes magnetically in order to obtain a homogenous population before freezing.
3. Determine cell number.
4. Transfer desired cell number into a 15 mL conical tube. Calculate with 5×10^6 cells per 250 μL aliquot.
5. Centrifuge for 5 minutes at $200\times g$.
6. Resuspend the cell pellet in StemMACS Cryo-Brew to 2×10^7 cells per mL.
7. Quickly transfer the cell suspension into suitable cryogenic vials (250 μL per vial).
8. Place the vials into an isopropanol freezing container and immediately store at -80°C .

9. After 24 hours transfer cells into a liquid nitrogen tank for long term storage.
10. For thawing of iPSC-derived cardiomyocytes follow the protocol for thawing of human iPSCs, step 1–6. Seed 300.000 cells/ cm^2 in appropriately coated cell culture plates.

Refer to www.miltenyibiotec.com for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

The Miltenyi Biotec logo and StemMACS are registered trademarks or trademarks of Miltenyi Biotec B.V. & Co. KG and/or its affiliates in various countries worldwide. All other trademarks mentioned in this publication are the property of their respective owners and are used for identification purposes only.

Copyright © 2023 Miltenyi Biotec and/or its affiliates. All rights reserved.