Miltenyi Biotec

StemMACS™ Cryo-Brew

Order no. 130-109-558

Contents

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 Ca^{2+} and Mg^{2+}.

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 10^6 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 10^6 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.

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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×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 (7000–16,000 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×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.

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