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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.

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 ($7000\text{--}16,000\text{ cells/cm}^2$) 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.

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