Protocol: Thawing and culturing StemRNA human iPSCs

Overview

This protocol describes procedures thawing human StemRNA™ iPSCs (Cat. No. RCRP004N, RCRP005N) that have been cryopreserved using NutriFreez D10 Cryopreservation Medium (formerly called CryoStem™ Freezing Medium).

Caution

This protocol uses cells that have been stored in liquid nitrogen. Liquid nitrogen is a freezing hazard, and evaporation of liquid nitrogen can generate significant pressures that can rupture closed vessels. Please take appropriate precautions when working with these cells.

Required Materials

PRODUCT DESCRIPTION	CAT. NO.
Cryopreserved iPS Cells	RCRP004N RCRP005N
NutriStem® hPSC XF Medium Growth medium that the cells were cultured in prior to cryopreservation	01-0005
Growth plate or dish An appropriate growth plate coated with the matrix that the cells were cultured on prior to cryopreservation.	Supplied by end user
iMatrix-511 Growth matrix that cells were cultured on prior to cryopreservation	NP892-02

Store all required materials according to the manufacturer's recommendations.

Thawing Protocol

Note: Have on hand NutriStem hPSC XF Medium that the cells were originally cultured in and an appropriate dish or plate coated with the growth matrix that the cells were originally culture on (iMatrix-511; see: https://www.reprocell.com/downloads/1536829846ReproCELL-STG-Protocol-STG_StemRNA-NM-iMatrix-Protocol-Final-June-2_2016.pdf) .

1. Briefly warm NutriStem® hPSC XF Medium in a 37 °C water bath.
2. Add 9 mL of warmed NutriStem® hPSC XF Medium to a centrifuge tube.
3. Rapidly thaw the cryovial of cells in a 37 °C water bath by gently shaking the vial and remove the vial when only a small frozen cell pellet remains. Do not vortex cells.

4. Disinfect the vial by wiping it down with a cloth moistened with 70% ethanol or isopropanol.

5. In a sterile biological safety cabinet, transfer the contents of the cryovial drop by drop into the 9mL of culture medium in the previously prepared centrifuge tube. Gently rock to continually mix the cells as the new cell droplets are added to the tube.

6. Centrifuge the cells at 200 x g for 5 minutes. Remove and discard supernatant.

7. Gently resuspend the cell pellet in NutriStem® hPSC XF Medium (Cat. No. 01-0005, and plate on an iMatrix-511 coated surface at the desired plating density (described in lot data sheet). Incubate the plate at 37 °C.

8. Refresh culture medium 48 hr after plating.

Culture and Passaging

Human iPSC cultures should be monitored and cared for every day, as the overall quality of the culture can change rapidly. Human iPSCs are generally passaged every 4 to 7 days in culture, but the actual passaging schedule and split ratio for each passage will vary depending on the cell culture's quality and growth rate. Within the first few days of each passage, the proliferating cells grow easily in a monolayer colony. Once the colony becomes large, the proliferating cells begin to pile up, sometimes causing unwanted spontaneous differentiation to occur. It is important to passage the cells before the cultures become overgrown.

For maintenance and expansion, the iPSCs should be cultured in NutriStem Medium on iMatrix-511 or adapted to other proven human iPSC culture conditions. Between passages, the cell culture medium should be exchanged every day to provide necessary growth factors for the maintenance of human iPSCs.

For the first passage or two after recovery from cryopreservation, the cells should be passaged manually using the EDTA passaging method. The cells can be passaged using an EDTA only or enzymatic protocol after that.

REPROCELL Inc (Japan)

KDX Shin-yokohama 381, Bldg.9F
3-8-11, Shin-yokohama,
Kohoku-ku, Yokohama,
Kanagawa 222-0033,
Japan

Tel: +81 45 475 3887
Email: info-asia@reprocell.com

REPROCELL USA Inc

9000 Virginia Manor Road
Suite 207
Beltsville, MD 20705
USA

Tel: +1 301 470 3362
Email: info-us@reprocell.com

REPROCELL Europe Ltd

Thomson Pavilion,
Todd Campus,
West of Scotland Science Park,
Acre Road,
Glasgow G20 0XA, UK

Tel: +44 (0)141 465 3460
Email: info-emea@reprocell.com

www.reprocell.com