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 the 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
Y27632 Optional addition to help cells recover from thawing.	04-0012

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

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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).

Optional: For best recovery of thawed cells, supplement the NutriStem hPSC XF Medium with Y27632 (10 µM final concentration) during the thawing and plating steps. Unsupplemented NutriStem hPSC XF Medium should be used starting at the first medium change.

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. Using a 2 mL or 5 mL pipette, in a sterile biological safety cabinet, transfer the contents of the cryovial drop by drop into the 9 mL 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.

   Note: Do not use a 1000 µL or smaller pipette tip for transferring the cells to avoid disrupting the cell clusters.
6. Centrifuge the cells at 200 x g for 2 minutes. Remove and discard the supernatant.
7. Using a 2 mL or 5 mL pipette, 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 the lot datasheet). Incubate the plate at 37 °C.

   Important**:** Do not disturb the plate during the first 18-24 hr after plating to avoid detaching the cells from the plate. Do not examine the cells under the microscope until at least 24 hr after plating.
8. Refresh culture medium 24-48 hr after plating, depending on the culture vessel and the seeding density.

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 hPSC XF 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.

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