Protocol

iMatrix-511 Cell Culture Substrate: Use with NutriFreez D10 Cryopreservation Medium and NutriStem hPSC XF Medium

Overview

This protocol describes procedures for thawing human iPSCs that were cryopreserved in NutriFreez D10 Cryopreservation Medium (01-0020-50). This includes our catalogue StemRNA™ iPSCs.

We recommend that a hypoxic incubator (5% CO_2, 5% O_2) be used for plating cells.

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.

Abbreviations

PBS	Phosphate-buffered saline

Products

PRODUCT DESCRIPTION	CAT. NO.	FORMAT	STORAGE
Cryopreserved iPSCs	Provided by end user		LN2
NutriStem® hPSC XF Medium	01-0005	500 mL	–20 °C
Growth plate or dish	Provided by end user		
iMatrix-511	NP892-011	350 µg	4 °C
Stemolecule™ Y27632	04-0012	2 mg	4 °C

Protocol

1. Prepare an appropriate amount of **Plating Medium** composed of:
   a. NutriStem® hPSC XF Medium
   b. iMatrix-511MG (1.125 µg/mL final concentration)
   c. Y27632 (10 µM final concentration; from a 10 mM stock solution in DMSO)

Table 1: Recommended volumes of iPSC culture medium and reagents.

Vessel	Approximate Growth Area (cm^2)	NutriStem hPSC XF Culture Media Volume	0.5 mg/mL iMatrix-511MG	Wash Reagent ($DPBS^{-/-}$)	0.5× CT0.5× CTS TrypLE Select Enzyme S TrypLE Select Enzyme	10 mM Rock inhibitor Y27632 stock solution (Final concentration 10 µM)
100 mm	55	10 mL	22.5 µL	5 mL	5 mL	10 µL

2. For one 100 mm dish, add 9.5 mL **Plating Medium** to the dish.
3. Incubate the dish with medium in the hypoxic incubator for at least 30 min before thawing the cells.
4. Prewarm 10 mL NutriStem hPSC XF Medium to a 50 mL centrifuge and prewarm in a 37 °C water bath.
5. Remove the vial of cells from the liquid nitrogen tank and keep on dry ice for transfer to the laboratory.
6. Quickly thaw the cryovial of cells in a 37 °C antibacterial water bath for 3 minutes by gently swirling the vial until 2/3 of the frozen cell solution has melted. Do not vortex cells.
7. Disinfect the vial by wiping it with a cloth moistened with 70 % ethanol or isopropanol.
8. Add 0.5 mL of pre-warmed NutriStem® hPSC XF from step 4 in a drop wise manner to the cryovial.
9. Using a P-1000 pipette, gently transfer the entire volume (~1.5 mL) to a 15 mL tube. Use 1 mL of pre-warmed NutriStem® hPSC XF to gently rinse cryotube.
10. Slowly add to the tube the 1 mL of pre-warmed NutriStem® hPSC XF over a period of about 10 seconds, while gently tapping the 15 mL tube to mix.
11. Let stand for about 1 minute.
12. Again, slowly add 5.5 mL of pre-warmed NutriStem® hPSC XF over a period of about 10 seconds, while gently tapping the tube to mix. Final volume of cell suspension should be ~8 mL.

Note: This sequential slow addition of culture medium is required to avoid severe osmotic shock to the cells.

13. Centrifuge the tube at 300 × g for 5 minutes at room temperature.
14. Spray the outside of the tubes with 70 % ethanol before transferring back into the BioSafety Cabinet (BSC), being careful not to disrupt the cell pellet.
15. Discard the supernatant.

16. Gently resuspend the cell pellet in the tube in 1.5 mL of pre-warmed, equilibrated NutriStem® hPSC XF, making sure to not pipette more than twice.
17. Put aside 20 µL of the cell solution in a 1.5 mL microcentrifuge tube.
18. Using the three 100 mm dishes prepared in step 2, add 0.5 mL of the cell suspension to each dish containing 9.5 mL Plating Medium for a total volume of 10 mL culture medium. Gently rock each dish from side to side and back and forth several times to evenly distribute the cells in the dish.
19. Place the dishes into the hypoxic incubator set at 37°C, 5% CO_2, and 5% O_2.
20. Count the reserved 20 µL cell suspension and calculate the live cell density. This reflects the viability.

Note: The cells are counted in step 20 to reduce the time out of the incubator. Alternatively, count the cells after Step 18 and adjust plating density as desired (plating density recommended at thaw: ~3-4 × 10^5 per 100 mm dish or 6-7 × 10^3 cells/cm^2).

Important: Do not disturb the dishes during the first 18-24 hours after plating to avoid detaching of cells from the dish. Examine the cells under the microscope ~24 hours after plating.

21. Change NutriStem® hPSC XF on the dishes every day thereafter XF without Y27632 or additional iMatrix (e.g., for regular medium changes use 10 mL NutriStem hPSC XF per 100 mm dish).

Note: Cells will be ready for passaging when they present tightly packed appearance and a well-defined edge and the culture reaches approximately 70-80 % confluency, which generally occurs around 5 to 6 days post thaw. We recommend manual EDTA passaging for the first two passages. After that, EDTA or enzymatic passaging can be used.

Regulatory Disclaimer

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