Expansion of iPSCs

Upon reaching a desired confluence cells can be passaged into daughter plates and/or cryopreserved.

Reagents

Thiazovivin, Stemgent, #04-0017

mTeSR1 Feeder Free Media, Stemcell Technologies, #05850

HESC qualified Cultrex™, Trevigen Inc, #3434-001-02

DMEM/F12, Life Technologies, #10565-018

StemPro Accutase, Life Technologies, A11105-01

Synthafreeze, Life Technologies, A12542-01 (if freezing cells also)

Equipment

Centrifuge

Conical Tubes (15 mL), Thermo Scientific, #339651

6-Well cell culture dishes, Corning, #3516

Cryovial of choice

Protocol

All steps should be performed under conditions standard for sterile tissue culture work.

Plate Coating

For coating of dishes, please follow manufacturers recommended protocol. NYSCF typically uses a standard dilution of 1:100 of stock Cultrex™ (or Geltrex, licensed by Trevigen) following lot-to-lot testing. We recommended the coating of 6 well plates with 2 mL of Cultrex™ per well. A full 6 well plate coated in Cultrex™ will be required for iPSC expansion.

Expansion of iPSCs (per cell line)

1) Pre-warm DMEM/F12 (2mL per well, see below) and mTeSR1 with 1 µM Thiazovivin (final concentration) (enough mTeSR1 mLs for cell count re-suspension and final passage distribution into multiple wells of a 6 well plate based on 300-400k cells per well) to 37˚C.
2) Pre-warm Accutase (500 µL / well in a 12 well plate) to 37˚C.
3) Pre-warm Cultrex™ coated dishes for at least one-hour prior to use.
4) Aspirate culture medium from culture dish and add 500 µL / well of Accutase (in a 12 well plate).
5) Incubate at 37˚C/5% CO2 for 5-10 minutes (or until cells are freely floating).
6) Neutralize Accutase with 1 mL/well DMEM/F12.
7) Transfer the total volume (1.5 mL) to a 15 mL conical tube and rinse the well once with 1 mL of additional DMEM/F12.
8) Centrifuge cells at 800g for 4 minutes.
9) Aspirate the supernatant without disturbing the pelleted cells and re-suspend the pellet carefully in 2 mL of mTeSR1.

10) Determine the total number of cells and percent viability using an automated cell counter or a hemocytometer, cell counter and Trypan Blue exclusion (optional).
11) Calculate the volume of media that you need to dilute the culture down to the recommended seeding density. NYSCF recommends seeding 300,000-400,000 cells in a final volume of 2mL/well of a 6 well plate.
12) If freezing cells, re-suspend cells in Synthafreeze at a maximum density as recommended by the manufacturer and slow freeze before transferring cryovials to liquid nitrogen.