ALLEN INSTITUTE for CELL SCIENCE

CORIELL INSTITUTE FOR MEDICAL RESEARCH

Allen Cell Collection hiPSCs: Critical Starting Points

For lines labeled "AICS" ONLY. Not to be used on parental line GM25256 or other collection cell lines.

This document outlines critical points for the cell growth and health of the Allen Cell Collection of fluorescently tagged hiPSCs. Full protocols are available on our website, ***allencell.org****, or through* ***coriell.org/AllenCellCollection***

Before you begin...

Please download the Cell Line's ***Certificate of Analysis*** through Coriell's website (link above)

How should the Allen Cell Collection hiPSCs be stored?

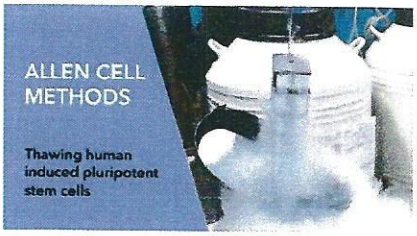
- Store vial(s) in liquid nitrogen (vapor or liquid phase) upon receipt

What reagents are required for the Allen Cell Collection hiPSCs?

- mTeSR-1 complete media
- mTeSR-1 complete media supplemented with 10 µM ROCK inhibitor (RI) (molecule Y-27632)
- Matrigel-coated 100 mm TC plates (Matrigel diluted 1:30 in DMEM/F12)
- Accutase

bit.ly/AllenCellVideos

How should the Allen Cell Collection hiPSCs be thawed?



- Thaw entire vial contents into a conical tube with 5 mL mTeSR-1 with 10 µM RI
- Pellet thawed cells to remove residual freezing media; re-suspend in mTeSR-1 with 10 µM RI
- Transfer re-suspended cells to 100 mm, Matrigel-coated TC plate prepared with 10 mL mTeSR-1 with 10 µM RI
- After 24 h change media to mTeSR-1 complete media (no RI); change media every 24 h
- Total time from cells thawed to cells re-suspended should be less than 10 minutes *(for this reason, only thaw 1-2 vials at a time)*

How should the Allen Cell Collection hiPSCs be passaged?

- These cells do best with single cell passaging using Accutase
- Cells should be spun out of Accutase and re-suspended in mTeSR-1 with 10 µM RI
- Plate cells on fresh Matrigel-coated plates or dishes
- Plate cells in mTeSR-1 with 10 µM RI for the first 24 h, then feed mTeSR-1 complete media (no RI) every 24 h until desired confluence is reached (70-85% confluence)

How should the Allen Cell Collection hiPSCs be imaged?

- The tagged proteins are expressed endogenously and therefore may not appear as bright as they would in an overexpressed system
- Best practices include plating on glass coated with phenol red-free Matrigel, using phenol red-free mTeSR-1 complete media

Immediate questions? *Ask the community at forum.allencell.org*

How should the Allen Cell Collection hiPSCs be cryopreserved?

- hiPSC should be passaged as single cells for freezing in cryovials using Accutase
- Use a freezing medium made of mTeSR-1, Knockout Serum Replacement, and 10% DMSO
- Freeze between 2.5 x 10^5 to 1 x 10^6 cells per 500 µL freezing medium per vial
- Use a "Mr. Frosty" or similar device for slow cooling at -80°C for up to 48 h
- Store cells in liquid nitrogen (liquid or vapor phase) for long term storage (>48 h)

Full, detailed protocols for each of these steps can be found at https://www.allencell.org/methods-for-cells-in-the-lab