ALLEN INSTITUTE for CELL SCIENCE

Allen Cell Collection: Getting Started

Critical Points

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

Store vials in liquid nitrogen upon receipt (liquid or vapor phase).

Thaw one (1) vial of cells into one (1) 100 mm TC plate. Do not thaw into a 6-well plate.

- Coat the TC plate with Matrigel diluted 1:30 in DMEM/F12.
- Use mTeSR-1 complete media with 10 µM ROCK inhibitor (molecule Y-27632) and 1% (v/v) penicillin-streptomycin.

Passage cells initially as single cells using Accutase.

Details below. Thawing this cell line in a different manner may result in decreased viability.

Thaw*

- Thaw one vial of cells and transfer the contents to a conical vial containing 5 mL mTeSR-1 with 10 µM ROCK inhibitor (RI) and 1% pen-strep.
- Pellet thawed cells and aspirate media; resuspend pellet in 2-3 mL of mTeSR-1 with RI and pen-strep.
- Evenly distribute the cells in a 100 mm, Matrigel-coated TC plate containing 10 mL mTeSR-1 with RI and pen-strep.
- After 24 h change media to mTeSR-1 complete media with pen-strep (no RI); change media every 24 h.
- From thaw to plating, the elapsed time should be less than 10 minutes *(for this reason, thaw 1-2 vials at a time).*

Passage*

- These cells have been acclimated to single cell passaging using Accutase.
- After dissociation, spin cells out of Accutase and resuspend in mTeSR-1 with 10 µM RI and 1% pen-strep.
- Plate cells in Matrigel-coated TC vessels. In our experience, vessels coated no more than 2 weeks in advance work best.
- Plate cells in mTeSR-1 with 10 µM RI and 1% pen-strep for the first 24 h, then feed with mTeSR-1 complete media with pen-strep (no RI) every 24 h until 70-85% confluency is reached.

Image

- 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 and using phenol red-free mTeSR-1 complete media.

Freeze (cryopreservation)

- This cell line should be dissociated into single cell suspension for cryopreservation. We recommend Accutase.
- Use a freezing medium made of mTeSR-1/RI/pen-strep, Knockout Serum Replacement, and DMSO in a 6:3:1 ratio.
- Freeze between 2.5 x 10^5 to 1 x 10^6 cells per 500 µL freezing medium per cryovial.
- 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).

Download complete written protocols at **allencell.org** or **coriell.org/AllenCellCollection**

*Watch our video protocols at **bit.ly/AllenCellVideos** or scan this QR code: