Product Specification Sheet

Product Name	**EZStem™ Freezing Medium**
Description	EZStem™ Freezing Medium was developed to maintain xeno-free conditions during cryopreservation when culturing human embryonic stem (ES) and induced pluripotent stem (iPS) cells in a xeno-free and feeder-free environment. It is a ready-to-use solution for cryopreservation of human ES/iPS cells. Human pluripotent stem cells preserved with EZStem™ Freezing Medium result in high cell viability and recovery and express typical pluripotent markers after thawing. EZStem™ Freezing Medium has been tested on both human ES and iPS cells. Better results were obtained in comparison with both serum-containing freezing media as well as competing serum-free products, making this an ideal product for cryopreservation of valuable human pluripotent stem cells.
Catalog Number	M050
Size	50 ml
Shipping	Ambient Temperature
Storage and Stability	Store at 4° C upon receiving. This product is stable for 6 months when stored as directed. Thaw this product on ice before use.
Quality Control	Human ES cells were frozen using EZStem™ Freezing Medium, thawed, cultured, and AP positive colonies were counted. When thawed and cultured, cells preserved with EZStem™ Freezing Medium had a minimum of 10% more AP positive colonies than an alternative freezing method.
Restricted Use	For Research Use Only. Not for use in diagnostic or therapeutic procedures.

ALSTEM
CELL ADVANCEMENTS
2600 Hilltop Drive, B/C 328, Richmond, CA 94806
www.alstembio.com Tel: (510) 708-0096 Email: info@alstembio.com

Protocol (M050)

Cryopreservation of human ES/iPS cells using EZStem™ Freezing Medium

Overview

This protocol can be used for the cryopreservation of human pluripotent stem cells cultured with feeder cells or in feeder-free conditions. The procedure describes the cryopreservation of cells cultured in one well of a 6-well plate. Amounts can be scaled up if freezing multiple wells; however, only 1 ml of cell suspension should be aliquoted into each cryogenic vial. Keep EZStem™ Freezing Medium on the ice at all times.

Procedure

Cryopreservation

1. Prepare EZStem™ Freezing Medium on ice.
2. Culture the cells in a 6-well plate until 60% to 80% confluency.
3. Aspirate medium from the hES/hiPS cell culture and rinse with DPBS (2 ml/well).
4. Add 1 ml per well of EZStem™ Enzyme-Free Stem Cell Dissociation Solution (ALSTEM, Cat. # M100) or other dissociation solution, such as accutase. Let it stand at room temperature for 1-2 minutes.
5. Aspirate Dissociation Solution, and gently rinse each well 2-3 times with 2 ml of DMEM/F-12 per well.
6. Add 2 ml/well fresh culture medium and scrape colonies off with a cell scraper.
7. Transfer the detached cell suspension to a 15 ml conical tube.
8. Centrifuge at 200 x *g* for 5 minutes at room temperature.
9. Gently aspirate the supernatant and loosen the cell pellet by tapping the bottom of the tube.
10. Gently resuspend the pellet in cold EZStem™ Freezing Medium, taking care to leave the clumps larger than would normally be done for passaging.

    Note: Adding ROCK inhibitor (Y-27632) to a final concentration of 10 µM to the freezing medium will enhance the recovery rate of human ES/iPS cells.

11. Transfer 1 ml of cell suspension into each labeled cryogenic vial.
12. Place vials into an isopropanol freezing container and place the container at -80° C overnight.
13. Transfer to a liquid nitrogen tank the next day.

Thawing Cryopreserved Human ES/iPS Cells

1. Coat the plate with Matrigel for 1 hour at 37° C.
2. Quickly thaw the human ES/iPS cells in a 37° C water bath by gently shaking the cryovial continuously until half thawed. Remove the cryovial from the water bath and spray with 70% ethanol to sterilize.

3. Transfer the contents of the cryovial to a 15 ml conical tube. Add 10 ml warm mTeSR1 dropwise to the tube, gently mixing as the medium is added.
4. Centrifuge cells at 200 x *g* for 5 minutes at room temperature.
5. After centrifugation, aspirate the medium from 15 ml tube.
   Note: Remove the freezing medium as much as you can. Too much residual freezing medium will decrease the recovery rate.
6. Gently resuspend the cell pellet in 2 ml mTeSR1 containing 10 µM ROCK inhibitor (Y-27632), taking care to maintain the cells as small cell clumps.
7. Remove the Matrigel solution from a coated tissue-culture 6-well plate. Transfer the medium containing the cell clumps to the Matrigel-coated 6-well plate.
8. Place the plate into the 37° C incubator and move the plate in quick side-to-side, forward-to-back motions to evenly distribute the clumps within the wells. Culture the cells at 37° C, with 5% CO_2 and 95% humidity.
9. Change medium daily. Check for undifferentiated colonies that are ready to passage when colonies are big enough (approximately 5-7 days after thawing).