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	Dry ice
Storage and Stability	Store at -20 °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.

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Protocol

Cryopreservation of human ES/iPS cells using EZStem Freezing Medium

OVERVIEW

This protocol can be used for the cryopreservation of human embryonic stem (hES) 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 aliquotted into each cryogenic vial. Keep EZStem Freezing Medium on ice at all times.

CRYOPRESERVATION PROCEDURE

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 0.5 mL per well of EZStem Enzyme-Free Stem Cell Dissociation Solution (cat. no. M100, ALSTEM). 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.

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.

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