ProMab Biotechnologies, Inc.

Protocol

Cryopreservation of human ES/iPS cells

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™ (cat. # M050, ProMab Biotechnologies)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% confluent.
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. # M050, ProMab Biotechnologies). 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.