Protocol

Cryopreservation of human ES cells using CryoStem 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 CryoStem Freezing Medium on ice at all times.

Product Description	Cat. No.	Format	Storage
CryoStem™ Freezing Medium	01-0013-50	50 ml	4°C

ADDITIONAL MATERIALS REQUIRED

- Dispase (Invitrogen)
- DMEM/F-12 Medium
- 15 ml conical tubes
- Cryogenic vials
- Isopropanol freezing container

MATERIAL PREPARATION

- **Dispase Solution**

Dissolve Dispase in DMEM/F-12 medium to a concentration of 1 mg/ml and filter sterilize with a 0.22 µm pore size filter unit. Dispase Solution can be stored at 4°C for up to 2 weeks.

> ***Note**: Cells may be detached using the enzyme and method that the cultures have been routinely passaged in.*

CRYOPRESERVATION PROCEDURE

1. Culture the cells in a 6-well plate until 60% to 80% confluency.
   > ***Note:** Cells should be frozen at their log-growth phase. Typically, this is around 4 days post-passaging.*
2. Aspirate the culture medium.
3. Add 1 ml of Dispase Solution.
4. Incubate at 37°C or at room temperature until the edges of the cell colonies begin to loosen from the plate. Incubation times will vary between cell lines, colony sizes, and enzyme used. Begin checking the culture after 3 minutes.
5. Carefully aspirate the Dispase Solution and add 2 ml of fresh cell culture medium into each well.
6. Using a 5 ml pipet, pipet the medium across the cells while gently scraping the cells from the plate.
7. Transfer the cell suspension to a 15 ml conical tube.

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8. Centrifuge at 200 x g for 5 minutes at room temperature.
9. Aspirate the supernatant and loosen the cell pellet by tapping the bottom of the tube.
10. Add 1 ml of **cold** CryoStem Freezing Medium.
    Note: *The CryoStem Freezing Medium is a 1X solution and should not be diluted before use.*
11. Gently mix 2 to 3 times to resuspend the cell pellet.
12. Quickly transfer the 1 ml of cell suspension into a cryogenic vial.
13. Place the vial into an isopropanol freezing container and transfer to -80°C. The isopropanol freezing container will freeze the vial at a rate of approximately -1°C per min.
14. The next day, transfer the vial to a liquid nitrogen tank for long-term storage.

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