Using the hES Cell Cloning & Recovery Supplement to Enhance Thawing and Recovery, Single Cell Cloning, and Passaging of hES Cells

Overview

The hES Cell Cloning & Recovery Supplement can be used to enhance cell recovery after storage in liquid nitrogen, after passaging, or for cloning hES cells from individual cells. This protocol was developed using the H1 embryonic stem (ES) cell line. If using another cell line, the optimal supplement concentration should be determined as its effects may vary between different cell lines and media.

Product Description	Cat. No.	Format	Storage
hES Cell Cloning & Recovery Supplement	01-0014-500	5 x 100 µl	4°C

Additional Materials Required

- Dispase (Invitrogen)
- DMEM/F-12 Medium
- 0.05% Trypsin/EDTA
- 15 mL conical tubes
- 50 mL conical tubes
- 6-well tissue culture plate
- 40 µm cell strainer
- Cryogenic vials
- Isopropanol freezing container

Material Preparation

Supplemented Medium

Thaw one vial of the supplement and dilute from the stock 1000X solution to a 1X working solution by adding an appropriate volume to the cell culture medium. Discard any remaining Supplemented Medium. Store any remaining supplement at 4°C. Multiple freeze/thaw cycles will not affect the activity of the supplement.

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: The optimal enzyme can vary depending on cell line and culture conditions. Cells may be detached using the enzyme and method that the cultures have been routinely passaged in.

Thawing and Recovery Procedure

1. Remove the vial of cells from the liquid nitrogen storage tank.
2. Roll the vial between gloved hands for 3 to 5 seconds to remove the frost.
3. Immerse the vial into a 37°C water bath.

Note: Do not submerge the cap of the vial in the water bath to prevent possible contamination.

4. When only a small ice crystal remains, remove the vial from the water bath and submerge briefly in ethanol.
5. In a sterile biological safety cabinet, transfer the contents of the vial directly to the bottom of a 15 mL conical tube.
6. Slowly add 9 mL of pre-warmed cell culture medium to the cells in the 15 mL tube. Adding medium slowly will reduce osmotic shock to the cells.
7. Centrifuge the cells at 200 X g for 5 minutes at room temperature.
8. Bring the pelleted cells back to the biological safety cabinet, and carefully aspirate the supernatant.
9. Gently resuspend the pellet in 2 mL of Supplemented Medium.
10. Transfer the cells to one well of a 6-well plate that has been treated appropriately. For example, cells may need to be plated on a feeder layer or on coated plates.
11. Incubate the cells at 37°C and 5% CO_2 overnight.
12. The next day, aspirate the medium and replace with cell culture medium without the supplement.

Growing Human Embryonic Stem Cells from Individual Cells

This protocol uses a 6-well plate format. Amounts may be scaled up or down if using another size format.

1. Culture the cells until they are 60 to 80% confluent.
2. Aspirate the cell culture medium and add 1 mL per well of 0.05% Trypsin/EDTA.

Note: CellMates™ Accutase® Cell Detachment Solution (Stemgent Cat. No. 01-0006), or CellMates™ Accumax® Cell Dissociation Solution (Stemgent Cat. No. 01-0007) can be used in place of Trypsin/EDTA.

3. Incubate at 37°C for 3 to 5 minutes or at room temperature for 5 to 8 minutes.
4. Add 3 mL per well of cell culture medium containing FBS or a Trypsin inhibitor to neutralize the Trypsin.
5. Using a 1000 µL tip, pipet the medium across the wells to detach the colonies. Pipet up and down 5 to 10 times to break up cell colonies into a single cell suspension.
6. Transfer the cell suspension to a 15 mL conical tube and centrifuge at 200 x g for 5 minutes at room temperature.
7. Aspirate the supernatant and loosen the cell pellet by tapping the bottom of the tube.
8. Resuspend with 2 to 5 mL per well of cell culture medium.
9. Place a 40 µm cell strainer into a 50 mL conical tube.
10. Add the cell suspension to the cell strainer. Clumps of cells will be retained while single cells and medium will pass into the conical tube.
11. When all of the suspension has passed through, remove the cell strainer from the 50 mL conical tube and discard.
12. Remove a sample of the single cell suspension to perform a cell count.
13. Centrifuge the single cell suspension at 200 x g for 5 minutes at room temperature.
14. While the cells are spinning, determine the cell number and calculate the amount of the medium needed to achieve the desired cell density.
15. Aspirate the supernatant and loosen the cell pellet by tapping the bottom of the tube.
16. Resuspend the cells with the appropriate amount of Supplemented Medium.
17. Plate the cells at the desired density onto the appropriate plate. For example, cells may need to be plated on a feeder layer or on coated plates.
18. Incubate the cells at 37°C and 5% CO_2 overnight.
19. The next day, aspirate the medium and replace with cell culture medium without the supplement.
20. Change the medium daily until cells are ready to be passaged.

Passaging Procedure

This protocol uses a 6-well plate format. Amounts may be scaled up or down if using another size format.

1. Culture the cells until they are 60% to 80% confluent.
2. Aspirate the culture medium and add 1 mL per well of Dispase Solution.
3. 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.
4. Aspirate the Dispase Solution and add 3 mL of cell culture medium into each well.
5. Using a 5 mL pipet, pipet the medium across the wells to gently detach the cell colonies.
6. Transfer the cell suspension to a 15 mL conical tube.
7. Centrifuge at 200 x g for 5 minutes at room temperature.
8. Aspirate the supernatant and loosen the cell pellet by tapping the bottom of the tube.
9. Gently resuspend the pellet in an appropriate amount of Supplemented Medium.
10. Plate the cells on the appropriate plate. For example, cells may need to be plated on a feeder layer or on coated plates. Different cell lines will vary but should be split between a 1:3 and a 1:10 ratio.
11. Incubate the cells at 37°C and 5% CO_2 overnight.
12. The next day, aspirate the medium and replace with cell culture medium without the supplement.
13. Change the medium daily until cells are ready to be passaged.

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