hES Cell Culture using Stemedia NutriStem XF/FF Culture Medium

NutriStem™ XF/FF Culture Medium

Cat. No. 01-0005

Overview

This protocol outlines two procedures: transferring cultures from feeder-based to feeder-free conditions, and passaging cultures grown in feeder-free conditions with NutriStem medium. Both procedures were developed using the H1 human embryonic stem (hES) cell line. If using other pluripotent stem cell lines (including induced pluripotent stem cells), optimal conditions should be determined by the user. This protocol describes culturing cells in a 6-well format and splitting at a 1:6 ratio. Although a 1:6 split ratio is the most common when culturing in NutriStem, cells often require a different split ratio at each passage. The appropriate ratio is determined by observing the culture density and growth rate after each passage.

NutriStem Materials			
Name	Cat. No.	Size	Storage
NutriStem™ XF/FF Culture Medium	01-0005-100	100 mL	-20°C
NutriStem™ XF/FF Culture Medium	01-0005	500 mL	-20°C

Additional Materials Required

- Dispase (Invitrogen Cat. No. 17105-041)
- Collagenase, Type IV (Invitrogen Cat. No. 17104-019)
- Matrigel™ hESC-Qualified Matrix (BD Cat. No. 354277)
- DMEM/F-12 medium
- 0.22 µm pore size filter units
- 6-well tissue culture plates 15 mL
- conical tubes

Material Preparation

- Preparation of NutriStem Medium: Thaw NutriStem XF/FF Culture Medium at room temperature or overnight at 4°C. Thawed NutriStem Medium is stable for 2 weeks when stored at 4°C. NutriStem Medium must be warmed to room temperature before use. To ensure the stability of the medium, only warm the amount needed.
- Preparation of 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.
- Preparation of Collagenase Solution: Dissolve Collagenase Type IV in DMEM/F-12 medium to a concentration of 1 mg/mL and filter sterilize with a 0.22 µm pore size filter unit. Collagenase Solution can be stored at 4°C for up to 2 weeks.
- Preparation of Matrigel-Coated Plates: Coat a 6-well tissue culture plate with Matrigel according to the manufacturer's instructions. If plates have been stored at 4°C, move to room temperature 1 hour prior to use.

Transferring hES Cells from Standard Feeder-Based Culture into Nutristem Culture

1. Aspirate the culture medium from the well.
2. Add 1 mL of Collagenase Solution.
3. Incubate at room temperature until the edges of the cell colonies begin to loosen from the plate.

Note: Incubation times will vary between cell lines and colony sizes. Begin checking the culture after 3 minutes. Do not over-incubate the culture, as hES cells are sensitive to enzymatic stress.

4. Aspirate the Collagenase Solution and add 2 mL of pre-warmed NutriStem Medium.
5. Gently scrape and wash the colonies off with a 5 mL pipet.
6. Transfer the contents to a 15 mL tube.
7. Use another 2 mL of medium to wash the well. Transfer the contents into the same 15 mL tube.
8. Centrifuge at 200 x g for 5 minutes at room temperature.
9. While the cells are spinning, aspirate the solution from a pre-warmed 6-well Matrigel-Coated Plate.
10. Add 1 mL of NutriStem Medium to each well of the plate.
11. Carefully aspirate the supernatant from the 15 mL tube without disturbing the cell pellet. Loosen the cell pellet by tapping the bottom of the tube.
12. Gently add 9 mL of NutriStem Medium. Mix the contents by gently pipetting up and down with a 5 mL pipet.
13. Add 1.5 mL of the cell suspension into each well of the plate.
14. Place the plate into the 37°C incubator and move the plate from side to side (in both directions) several times to evenly distribute the cells.
15. Incubate overnight at 37°C and 5% CO_2.
16. Change the medium daily with fresh NutriStem Medium until the hES cell colonies are big enough to passage (about 5 days).

Note: The Stemgent® hES Cell Cloning & Recovery Supplement (Stemgent Cat. No. 010014) can be used to enhance cell recovery after transferring hES cells from feeder-based culture into NutriStem. Dilute the supplement in NutriStem Medium to a 1X concentration and add the supplemented medium to the cells during the first overnight incubation after seeding. When the medium is changed the next day, remove the supplement and continue with standard culturing.

Passaging hES Cells Grown in Nutristem on Matrigel

1. Aspirate the culture medium from the well.
2. Add 1 mL 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.

Note: Incubation times will vary between cell lines and colony sizes. Begin checking the culture after 3 minutes. Do not over-incubate the culture, as hES cells are sensitive to enzymatic stress and may lift from the plate during the wash step.

4. Aspirate the Dispase Solution and wash the well twice with 2 mL of sterile DMEM/F12.

*Note: DMEM/F-12 should be added **very gently** to avoid detaching the cells from the surface of the plate.*

5. Add 2 mL of pre-warmed NutriStem Medium to the well. Scrape and wash the colonies off with a 5 mL pipet.
6. Transfer the cell suspension to a 15 mL conical tube.
7. Use another 2 mL of medium to wash the well. Transfer the contents into the same 15 mL tube.
8. Gently add 5 mL of NutriStem Medium. Mix the contents by gently pipetting up and down with a 5 mL pipet.
9. Aspirate the solution from a 6-well Matrigel-Coated Plate.
10. Add 1 mL of NutriStem Medium to each well of the plate.
11. Add 1.5 mL of the cell suspension into each well of the plate.
12. Place the plate into the 37°C incubator and move the plate from side to side (in both directions) several times to evenly distribute the cells.
13. Incubate overnight at 37°C and 5% CO_2.
14. Change the medium daily with fresh NutriStem Medium until the hES cell colonies are big enough to passage (about 5 days).

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