Embryonic Stem (ES) Cells

1. **30 Minutes Prior to Handling Cells** – Pre-warm complete growth medium for ES cells at 37°C for at least 30 minutes before adding to cells.
2. **One Hour Prior to Thawing the ES Cells** – Perform a 100% medium change for the MEFs using complete growth medium for ES cells.
3. Thaw the vial of ES cells by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 90 seconds).
4. Remove the vial from the water bath before the contents are completely thawed, and decontaminate by dipping in or spraying with 70% ethanol.

**All of the operations from this point on should be carried out under strict aseptic conditions.**

5. Transfer the vial’s contents plus 5 mL of complete growth medium for ES cells to a 15 mL centrifuge tube. Use an additional 1 mL of media to rinse the vial and transfer the liquid to the 15 mL tube. Add 4
Spin the cells at 270 x g for 5 min. Aspirate the supernatant and resuspend the pellet in 2 mL of complete growth medium for ES cells.

Add the 2 mL of cell suspension to the appropriate size flask containing feeder cells and fresh complete growth medium for ES cells (see batch specific information). ES cells should be plated at a density of 30,000 – 50,000 cells/cm².

Incubate the culture at 37°C in a humidified 5% CO₂/95% air incubator.

Routine Handling
Perform a 100% medium change every day. Passage the cells every 1 to 2 days. If the colonies are close to or touching each other the culture is overgrown. Overgrowth will result in differentiation. Make sure that you have prepared a sufficient number of flasks pre-plated with MEF feeder layers to support frequent passage of the ES cells.

Subculturing Procedure

Note: To insure the highest level of viability, pre-warm media and Trypsin/EDTA to 37°C before adding to cells. Volumes used in this protocol are for T75 flasks. Proportionally adjust the volumes for culture vessels of other sizes. A split ratio of 1:4 to 1:7 is recommended.

Feeder Cell Preparation for Subcultures
1. Daily maintain a sufficient number of flasks that have been pre-plated with MEFs in complete medium for feeder cells.
2. One hour before subculturing the ES cells, perform a 100% medium change for the MEFs using complete growth medium for ES cells.

Dissociation and Transfer of ES Cells
1. Aspirate the medium from the flask(s) containing ES cells.
2. Wash with PBS Ca²⁺/Mg²⁺-free (ATCC 30-2200).
3. Add 3.0 mL of 0.25% (w/v) Trypsin/0.53 mM EDTA solution (ATCC 30-2101) and place in incubator.
   After about one minute the ES colonies will dissociate and all cells will detach from the flask.
4. Dislodge the cells by gently tapping the side of the flask then wash the cells off with 7-10 mL of fresh culture medium. Triturate cells several times with a 10 mL pipette in order to dissociate the cells into a single-cell suspension.
5. Spin the cells at 270 x g for 5 min. Aspirate the supernatant.
6. Resuspend in enough complete growth medium for ES cells to reseed new vessels at the desired split ratio (i.e. a split ratio of 1:4 to 1:7 is recommended). Perform a cell count to determine the total number of cells. ES cells should be plated at a density of 30,000 – 50,000 cells/cm².
7. Add separate aliquots of the cell suspension to the appropriate size flask containing feeder cells and add an appropriate volume of fresh complete growth medium for ES cells to each vessel.
8. Incubate the culture at 37°C in a humidified 5% CO₂/95% air incubator. Perform a 100% medium change every day, passage cells every 1-2 days.

Cryopreservation Medium
Complete growth medium supplemented with an additional 10% FBS and 10% DMSO

Comments
The deposited line has been shown to be germline competent.

References
References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 1
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the Biosafety in Microbiological and Biomedical Laboratories from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

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