

Mouse ES Cell Culture

General information on culturing ES cells.

All reagents used for the culturing of ES cells should be “tissue culture grade” or “tissue culture tested”. All procedures are to be done under sterile conditions in a laminar flow hood using sterile instruments and detergent-free glassware. Dulbecco’s modified Eagle’s media (DMEM) (Gibco #12100-046) is prepared for ES cell medium as described below. The plates must be specially treated before seeding the ES cells. This is performed either by coating the plates with 0.1-0.2% gelatin (Type B) (Sigma #G-9391) or preparing a layer of mitotically inactivated embryonic fibroblasts for the ES cells to grow on. MEFs can be purchased, treated or untreated, from Specialty Media. A vial of the untreated MEFs can be expanded and treated with Mitomycin C, used immediately or frozen down for future use.

ES cells should be fed every day and split every second day (approximately 70-80% confluency, colonies almost touching each other) using a 1:5 split ratio (this might need to be adjusted depending on the cell line). It is generally not recommended to grow ES cells for long periods of time in LIF alone (i.e., on gelatin-coated plates) without a feeder layer. If the cells grow to over-confluency, regardless of what they are growing on, it can result in their differentiation and loss of germline competence.

Media Preparation

Embryonic Stem Cell Medium (ES)

Dulbecco’s modified Eagle’s Medium (DMEM) with high glucose (Gibco #11960-044)

To the 500ml bottle add:

- 6mls of GlutaMAX, (GlutaMAX-1, Gibco 35050-061)
- 6ml of diluted β -mercaptoethanol, 100 μ M final**
- ****Note:** Diluted 2-mercaptoethanol is made by adding 70ul of 2-mercaptoethanol (Sigma, M7522) to 100ml of sterile PBS or water.
- 6mls of Sodium Pyruvate, 1mM final (Gibco, 11360-070)
- 6mls of Non-essential amino acids, 100 μ M final (Gibco, 11140-050)
- 1000U/ml of LIF (Leukaemia inhibitory factor, Chemicon ESG1107)
- penicillin/streptomycin (Gibco #15140-148, final concentration 50ug/ml each)

Plus the required amount of FBS for the different media types

-normal ES media has 15% which = 95ml of FBS added

NOTE: If you are thawing and passaging a Genetrap line that has a selection gene (G418, puromycin etc.) you need to add antibiotic to the media. (G418 = 165 ug/ml final, puromycin = 2ug/ml final)

Thawing Cells:

- Thaw the vial quickly in a 37°C water bath
- Wipe the vial with 70% ethanol
- Transfer the contents of the vial to a tube with at least 5mls of ES media in it
- Spin at 1000 RMP for 5 min
- Aspirate off the ES media
- Resuspend the pellet in 4ml of ES media
- Transfer contents to a 6cm tissue culture treated dish which has inactivated feeder cells on it
- Change the media the next day
- ES cells should be passaged every other day with a 1:5 split
- One 6cm dish can be passaged onto two 10cm dishes

Passage of ES cells on 10cm tissue culture plates:

- Remove the media from the plate.
- Rinse the plate with PBS (-Mg and Ca, Gibco cat# 14190-250), aspirate off the PBS
- Add 2mls of trypsin (Sigma T4049) to the plate.
- Incubate at 37°C for 5 minutes.
- After 5 minutes, add 3ml of media to the plate and resuspend the cells well.
- Dispense 1ml of cell suspension to each new plate distributing the cells well.
- Return the plates to the incubator.
- Feed the plates the next day.

Freezing Cells:

- ES cells can be frozen down using 50% complete DMEM, 40% FBS and 10% DMSO. Four 1ml vials of cells can be frozen from each 10cm plate of cells, 80% confluent (thaw back onto a 6cm plate).