ES cell culture protocol

Thawing SIGTR ES cells

1. Coat a single dish of a 6-well plate with 0.1% gelatin and aspirate off immediately before use.

!!!Warning: Loosen cap of cryovial immediately upon removal from liquid nitrogen or vials are likely to explode!!!

2. Thaw ES cells (vial contains approx. 2×10^6 cells, equivalent to 1/2 a confluent 6-well) in a 37°C water bath and add to 10 ml of pre-warmed ES cell medium**.** Mix well by swirling.
3. Pellet the cells by spinning for 3 minutes at 1200 rpm in a bench-top clinical centrifuge.
4. Aspirate off medium and gently resuspend cells in 5 ml of pre-warmed medium.
5. Transfer cell suspension to the 6-well plate and grow at 37°C in a humidified 7% CO_2 incubator.
6. Change medium the following day to remove dead cells and residual DMSO.

Passage and expansion of ES cell cultures

1. For a confluent well of a 6-well plate, aspirate medium off and wash two times with 5 ml of pre-warmed PBS.
2. Cover cells with 0.5 ml of 1× trypsin solution and return to 37°C incubator for 2 minutes or until cells are uniformly dispersed into small clumps.
3. Add 4.5 ml of medium to inactivate the trypsin.
4. Add 1 ml (~1×10^6 cells) to a freshly gelatinized 25 cm^2 tissue culture flask.

Freezing ES cells

1. Trypsinize a confluent 25 cm^2 flask of cells (approximately 1×10^7 cells) as described above.
2. Collect trypsinized cells in 9 ml of medium and pellet for 3 minutes at 1200 rpm.
3. Aspirate off medium and resuspend cell pellet in 2.5 ml of freshly prepared freezing medium. Aliquot 0.5 ml of cells into five cryotubes.
4. Freeze the vials at -80°C overnight and transfer to liquid nitrogen for long-term storage.