

Mouse ES cell revive, routine culture and freezing for long-term storage

Reagents required

ES cell culture medium:

In 450 ml knock-out DMED (BRL product), add:
80 ml Bovine serum for ES cell culture (BRL product)
5 ml Glutamax (BRL product)
5 ml Non-essential amino acid (BRL product)
5 ml Penicillin-Streptomycin (BRL product)
0.9 ml 2-mecaptoethanol (BRL product)
50 µl LIF (from 10^7 u/ml, BRL product)

Keep at -4°C .

1 X ES cell freezing medium:

For each 50 ml 1 X ES cell freezing medium, mix:
24 ml of ES cell culture medium (mentioned above)
20 ml Bovine serum for ES cell culture (BRL product)
6 ml DMSO (Sigma product)

Keep at -20°C till using.

ES cell reviving:

1. Coat 6-well plate (Corning product) with 0.01% Gelatin (prepared in PBS, Sigma product) at room temperature for 20 min.
2. Remove Gelatin from the plate. For D3H cell line, add 2×10^6 feeder cells (we use MEFs) for each plate. Incubate the plate at 37°C overnight. E14 cell line does not require feeder cells. Therefore, you can go directly to step 4.
3. Observe under microscope to make sure that the bottom of the well is completely covered by feeder cells.
4. Thaw cell line at 37°C . Add thawed cell line in a 15 ml centrifuge tube containing 8 ml ES cell culture medium. Spin at 4°C with 1,200 rpm for 5 min.
5. Remove the supernatant from the centrifuge tube and re-suspend ES cells into 3.5 ml ES cell culture medium.
6. Remove the medium from 3 wells of the 6-well plate coated with feeder cells. Add 0.5 ml, 1 ml and 2 ml of ES cells into each well. Add ES cells to wells to make total ES cell culture medium to 3 ml in total for each well.
7. Incubate the plate at 37°C .

Note: feeder cells can be coated into the plate simultaneously when ES cell is being revived.

ES cell routine maintenance:

1. Observe ES cells cell morphology and confluent under microscope to make sure that the ES cells are not differentiated. Change ES cell culture medium every day.
2. Every 3-4 days, split ES cells at least once.
3. 2-6 hours before split ES cells, change into fresh ES cell culture medium.
4. Incubate cells at 37°C.

ES cell long-term freezing:

1. Observe ES cells under microscope to make sure that your ES cells are about 85% confluent.
2. Change into fresh ES cell culture medium 2-6 hours before trypsinizing ES cells.
3. Wash wells containing ES cells to be frozen once with PBS.
4. Add 0.25% trypsin (BRL product) to cover the bottom of the cells. Incubate the plate at 37°C for 5 min.
5. Separate ES cells with thin-bore plastic pasture pipette to make sure that the cells are well separated.
6. Add 3 ml ES cell medium into each well and transfer ES cells into 15 ml centrifuge tube containing 5 ml ES cell culture medium.
7. Spin ES cell down at 4°C with 1200 rpm for 5 min.
8. Remove supernatant and re-suspend ES cells into 3 ml 1 X ES cell freezing medium mentioned before.
9. Aliquot 1 ml into each of 3 vials. Put each vial in the tray of the jar containing propane and keep jar in -80°C.
10. Within 1 month, transfer vials into liquid nitrogen for long-term storage.