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Embryoid bodies generation

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Guidelines

MATERIALS

Medium: NO LIF ES medium
Dish: BD Petri Dish.
Trypsin
4% PFA
PBS

Troubleshooting

Seed

1 Add Trypsin 2ml and gently shake plate

2 Add 8ml ES medium without LIF to inactivate trypsin

3 Centrifuge at 500rpm for 10min
00:10:00

4 Add 8ml of EB medium to each 10cm petri-dish while centrifuging

5 Gently resuspend pellet in 5ml EB medium by 5ml pipette, up and down for 5 times

6 Take 100ul cell suspension and dilute into 1ml. Count.

7 Take appropriate number of cells and dilute into 1×10^6 cells/ml

8 Gently re-suspend by 5ml pipette, up and down for 4 times

9 Seed 2ml of cells/dish

10 Save 2×10^6 cells for RNA extraction

Changing Medium

11 Transfer medium containing Ebs to 50ml tubes

Note

Medium should be changed every other day

12 Carefully add 5ml medium to dishes immediately and put back in incubator

Note

Try not to disturb the attached Ebs.

13 Sink cells in tubes by gravity for 5min

00:05:00

Note

It may need **longer** @ first time as EB is small at this time point.

14 Discard supernatant

15 Add 5ml medium to pellets

16 Mix samples by inverting a few times gently

17 Distribute samples to different dishes

Note

If Ebs settle down during distributing, invert tube gently for a few times again.

Outgrowth of Ebs

18 Prepare gelatin coated chamber slides (2 well)

19 Add 1.5ml of medium into each well

20 Transfer 1 dish of day4 Ebs into 15ml tube

21 Wait for Ebs sink to the bottom of tube by gravity for 5min

00:05:00

22 Discard supernatant

23 Add 10ml EB medium into pellets

24 Seed 0.5ml of EB to each well

25 Change medium everyday: suck supernatant, replace with fresh EB medium

Immunostaining

26 Take 2 wells for each group on day6, day8 and day12

27 Suck the medium carefully

28 Fix with 4% PFA for 30min @RT. (in hood)
00:30:00

29 Wash #1 with 2ml of PBS
00:05:00

30 Wash #2 with 2ml of PBS
00:05:00

31 Wash #3 with 2ml of PBS
00:05:00

32 Store samples in PBS, @ 4°C