Embryoid bodies generation

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Abstract

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Guidelines

MATERIALS

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

Protocol

Seed
Step 1.
Add Trypsin 2ml and gently shake plate

Seed
Step 2.
Add 8ml ES medium without LIF to inactivate trypsin

Seed
Step 3.
Centrifuge at 500rpm for 10min

DURATION
00:10:00

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

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

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

Seed

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

Seed

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

Seed

Step 9.
Seed 2ml of cells/dish

Seed

Step 10.
Save 2×10^6 cells for RNA extraction

Changing Medium

Step 11.
Transfer medium containing Ebs to 50ml tubes

NOTES

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Medium should be changed every other day

Changing Medium

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

NOTES

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Try not to disturb the attached Ebs.

Changing Medium

Step 13.
Sink cells in tubes by gravity for 5min

DURATION

00:05:00

NOTES

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

Changing Medium

Step 14.
Discard supernatant

Changing Medium

Step 15.
Add 5ml medium to pellets

Changing Medium

Step 16.
Mix samples by inverting a few times gently

Changing Medium

Step 17.
Distribute samples to different dishes

NOTES

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

Outgrowth of Ebs

Step 18.

Prepare gelatin coated chamber slides (2 well)

Outgrowth of Ebs

Step 19.

Add 1.5ml of medium into each well

Outgrowth of Ebs

Step 20.

Transfer 1 dish of day4 Ebs into 15ml tube

Outgrowth of Ebs

Step 21.

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

DURATION

00:05:00

Outgrowth of Ebs

Step 22.

Discard supernatant

Outgrowth of Ebs

Step 23.

Add 10ml EB medium into pellets

Outgrowth of Ebs

Step 24.

Seed 0.5ml of EB to each well

Outgrowth of Ebs

Step 25.

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

Immunostaining

Step 26.

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

Immunostaining

Step 27.

Suck the medium carefully

Immunostaining

Step 28.

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

DURATION

00:30:00

Immunostaining

Step 29.

Wash #1 with 2ml of PBS

DURATION

00:05:00

Immunostaining

Step 30.

Wash #2 with 2ml of PBS

DURATION

00:05:00

Immunostaining

Step 31.

Wash #3 with 2ml of PBS

DURATION

00:05:00

Immunostaining

Step 32.

Store samples in PBS, @ 4°C