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Growing iPSCs in eTeSR+

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We use this protocol and it's working

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Abstract

This protocol describes how to grow human iPSCs in eTeSR media. This protocol is based heavily on the protocol published by StemCell Technologies with minor modifications.

Plate Preparation

1 Dilute Matrigel in chilled DMEM according to lot instructions. Keep Matrigel on ice at all times.

2 Add 1 mL chilled diluted Matrigel to each well of 6-well plate using chilled serological pipettes. Use of chilled pipettes is essential.

3 Incubate plate at room temperature for 1 hour. Plates can be made in advance and stored at 4 ^{o}C sealed with parafilm for up to one week. Warm to room temperature for 1 hour prior to use.

Media Preparation

4 Combine eTeSR+ basal medium and supplement to make complete medium

5 Add CloneR 2 to eTeSR+ to make 10% solution (ex. 1.2 mL CloneR 2 in 10.8 mL eTeSR+)

6 Remove Matrigel and add 2 mL prepared media to each well of prepared plate

Cell Split

7 Wash well containing iPSCs with 2 mL room temperature D-PBS after removing media

8 Add 500 uL Accutase warmed to 37 ^{o}C to well

9 Place plate in 37 ^{o}C CO_2 incubator for 10 minutes

10 Add 1 mL room temperature eTeSR+ to well

11 Pipette 3 times slowly with 1000 uL pipettor

12 Transfer cell suspension to 15 mL tube

13 Centrifuge cell suspension at 300xg at room temperature for 5 minutes

14 Discard supernatant, add 1 mL eTeSR+ to tube

15 Flick tube 5 times to resuspend cells

16 Perform cell count

16.1 Add 10 uL cell suspension to 20 uL tube

16.2 Add 10 uL Trypan Blue to tube

16.3 Pipette 10 times with 20 uL pipettor to mix

16.4 Transfer 10 uL mix to each side of a Countess slide

16.5 Run slide on Countess

16.6 If cell count is lower than 1 million, re-centrifuge and resuspend in less than 1 mL of media, then recount; if cell count is greater than 10 million, add media, flick tube to mix and recount

17 Add 10,000-40,000 cells to each well of prepared plate with media

18 Move plate side-to-side and forward-to-back gently to distribute cells

19 Place plate in incubator

Feeding

20 Change media daily with room temperature eTeSR+

21 Cells are ready to split again when colonies have pale centers

22 Avoid going over 10 passages