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Regular maintenance of human pluripotent stem cells

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We use this protocol and it's working

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Abstract

This protocol describes the regular maintenance and passaging human iPSCs.

Attachments

mqjubj3up.docx
16KB

Materials

Reagents required

1. E8 Media (Gibco)
2. 0.5M EDTA (Gibco), working solution 0.5mM EDTA prepared in sterile PBS solution
3. Y-27632 Rock inhibitor (Tocris Bioscience)
4. Matrigel (corning)

Day 1

1 Pre coat a 6 well dish with Matrigel matrix for 24:00:00 or 1 hour. 1d

2 Thaw the frozen iPSCs by placing the vial in 37 °C water bath for 00:02:00 . 2m

3 After thawing, spray the tube with 70% ethanol and place in biosafety cabinet.

4 Aspirate the cells into 15 ml falcon tube and add 4 mL cold E8 media containing rock inhibitor.

5 Spin down the tube at 0.3 rcf, 00:03:00 . 3m

6 Aspirate the supernatant and resuspend in fresh E8 media containing rock inhibitor.

7 Remove Matrigel matrix and dispense appropriate number of cells onto Matrigel coated dishes.

Day 2

8 Remove the E8 media containing rock inhibitor and feed cells with E8 media without rock inhibitor.

9 Feed every day with fresh media till plate gets 60-70% confluency.

10 Cells were passaged every 5 days with E8.

iPSC passaging with 0.5 mM EDTA solution: Day 1

11 Pre coat a 6 well dish with Matrigel matrix for 24:00:00 or 1 hour. 1d

12 Remove the culture media.

13 Rince 1X with sterile PBS.

14 Add 1 mL of EDTA solution.

15 Place in the incubator for 5-7 minutes.

16 Bring the plate into biosafety cabinet and add 2 mL E8 media containing rock inhibitor.

17 Dispense all the cell suspension into 15 ml falcon tube.

18 Spin down the tube at 0.3 rcf, 00:03:00 . 3m

19 Aspirate the supernatant and resuspend in fresh E8 media containing rock inhibitor.

20 Remove Matrigel matrix and dispense appropriate number of cells onto Matrigel coated dishes.

iPSC passaging with 0.5 mM EDTA solution: Day 2

21 Remove the E8 media containing rock inhibitor and feed cells with E8 media without rock inhibitor.

22 Cells were passaged every 5 days with E8.