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IPSC Culture Methods (Updated) V.2

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We use this protocol and it's working

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Abstract

This protocol is a standard iPSC culturing protocol and based on common methods for culturing and maintaining iPSCs

Materials

Matrigel-Growth Factor Reduced (GFR) (Corning, #354230)
mTeSR Plus (StemCell Technologies, #100-0276)
Rock inhibitor (StemCell Technologies, #72304)
ReLeSR (StemCell Technologies, #05872)

Sterile tissue culture plate.
5 mL Seriological or Widebore p1000 tips.
Wet Ice.
Water bath.

Before start

This protocol assumes that the user has a basic understanding of sterile technique. Proper personal protective equipment must be worn at all times.

Material Preparation: Day -02 and Day -01

1 (Day -02) Place Geltrex/Matrigel and either a 2 or 5 mL serological in 4C. Allow basement membrane to thaw slowly.

2 (Day -02) Place mTeSR 5x supplement in 4C, allow it to slowly thaw. Do not place in heated water bath or bead bath.

3 (Day -01) While working on ice, use the serological to aliquot 0.5 mL of the Geltrex/Matrigel in pre-chilled vials. Freeze aliquots in -20C.

4 (Day -01): Create complete mTeSR media by adding thawed (yet cold) 5x supplement in roomtemperature mTeSR basal media. Keep 200 mL of media for Day 0, freeze the rest of the media. Keep thawed complete mTeSR media in 4C for 14 days. Frozen complete media is stable for 6 months.

5 (Day -01): Reconstitute ROCK inhibitor (Y27632) in 1x PBS to 50 mM.

6 (Day -01) Create a 10 µM ROCK inhibitor solution in complete mTeSR plus media (ROCK-media). For every vial to be thawed will need 10 mL of ROCK-media.

Thawing: Day 01

7 Thaw Geltrex/Matrigel aliquot in ice bucket or in 4C for 10-20 minutes.

8 To make coating solution, dilute Geltrex/Matrigel at a 1:100 ratio in chilled DMEM/F12 (e.g., 60 ul Geltrex for 6 mL DMEMF12).

9 Add 1 mL of coating solution per 9.6 cm^2 (i.e., 1 mL for one well of a 6-well plate)

10 Place the coated plate in a tissue culture incubator for 1 hour. Do not allow the coating solution to dry. If not used immediately, wrap the plate with parafilm and place in 4C.

11 Discard coating solution, add 2 mL of ROCK-Media per 9.6 cm^2. Incubate at 37C till used. Do not incubate at 37C without cells for more than 8 hours.

12 Collect cells from cold storage and place on dry ice. Do not allow the vials to thaw at room temperature.

13 Label conical tubes with vial contents.

14 Clean the vial with 70% ethanol. Bring the cell vial under the tissue culture hood, twist the vial to the point were any pressure is release. Reseal the vial before leaving the tissue culture hood.

15 While holding the vial in hand, submerge the vial in heated water bath and swirl the vial in a figure eight motion.

16 Check frequently to see if the frozen medium is beginning to melt. This process may take up to 1 minute. Small volumes (~500uL) will thaw within 1 minute. When there is only a small piece of ice floating in the cryovial, remove the vials from the water bath.

17 Place the vial in the hood after cleaning with 70% ethanol.

18 Transfer the cell suspension to the labeled sterile 15mL conical tube using a wide bore P1000 or serological. Quench the cell solution with 1 mL media, collect, and add to the cell conical tube gently. Add 7mL of ROCK-media drop-wise, then gently swirling the medium to reduce osmotic shock.

19 Centrifuge for 3 minutes at 300 × g. While the vials spins, label the coated plates with vial content.

20 When centrifugation is complete, return the conical tube to the hood after cleaning with 70% ethanol.

21 Discard the supernatant from the tube without disturbing the pellet.

22 Resuspend cells with the remaining 2 mL of ROCK-media and transfer the cells to the previously labeled 6 well plate.

Media changing: Day 01-08

23 Thaw complete mTeSR plus media at room temperature. Do not warm in heated bath.

24 Observe cells via microscope after 12-24 hours of plating. Document morphology of the cells and confluency of the well.

25 Media change- with equal parts media- with wells containing attached cells. Add equal parts media to wells that have no attachment. Attachment can occur after 72 hours of plating. If no attachment has occured, discard those wells and rethaw.

26 Media change cells every other day using the parameters. On Friday- or when two consecutive days of maintenance is not possible- perform a double feed cells by multiplying the values below by 2x.

If confluency is <40%, media change with 2 mL
If confluency is 40-60%, media change with 3 mL
If confluency is >60%, media change with 4 mL (you should be passaging the cells at this point.

Passaging with ReLeSR

27 Warm complete mTeSR plus media to room temperature. Do not place in heated bath.

28 Have pre-coated Matrigel/Geltrex plate ready.

29 Remove spent media, wash cells with 1x PBS, and add 1 mL of ReLeSR per 9.6 cm^2. Leave the solution on the cells for ~30 seconds, then discard the solution.

30 Keep the wells dry at room temperature for 4-5 minutes.

31 Add 1 mL/ 9.6 cm^2 of complete mTeSR plus to the cells. Observe if cells "peel off", if no "peeled off clumps" are not visible, gently tap the side of the tissue culture plastic to release cells.

32 Using a serological or wide bore pipette, mix 3-4 times gently. Do not introduce bubbles.

33 Transfer cells to the pre-coated tissue culture plate.