NOV 17, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.n92ldmj99l5b/v1

Protocol Citation: rachel.bates, Matthew Gegg 2023. Culturing iPSC cells in Essential 8 medium. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ldmj99l5b/v1

Created: Nov 17, 2023

Last Modified: Nov 17, 2023

PROTOCOL integer ID: 91083

Culturing iPSC cells in Essential 8 medium

Matthew
rachel.bates[1], Gegg[1]

[1]UCL

rachel.bates

ABSTRACT

Culturing of iPSC lines in essential 8 medium, thawing and passaging techniques.

MATERIALS

Essential 8 media (Thermo, Cat # A1517001).
Vitronectin (Thermo, Cat# A14700)

Keywords: ASAPCRN

Funders Acknowledgement:
ASAP

Preparing plates and medium for cells

1 Essential 8 media (Thermo, Cat # A1517001). This consists of 500 mL of basal medium and 10 mL of supplement. Media with supplement has a shelf life at 4 °C for 2 weeks. Unless culturing large numbers of cells it is best to make up 100 mL aliquots (or whatever volume you think you will require over two weeks).

2 Defrost 10 mL of supplement in fridge overnight. Aliquot in to 2 mL aliquots and store at -20 °C. Never defrost supplement in water bath.

3 Mix 2 2 mL ml supplement with 98 mL l E8 media.

4 Warm up complete media at room temperature , not a water bath.

5 Stock vitronectin is 500 □g/ml. Require 5 □g/ml to coat plates therefore dilute stock 1/100 in PBS. Vitronectin (Thermo, Cat# A14700)

6 Need 1 mL vitronectin solution/6 well (scale up or down as required for different sized dishes: need ~ 5 □g/cm2).

7 Incubate at room temperature for 1 hour. The plates can then be used immediately or wrapped up in cling film and stored in fridge for one week. Do not let dry them out. Plates need to be warmed up to room temperature before cells are seeded in them. Aspirate away vitronectin solution just prior to

seeding cells. No need for wells to be washed with PBS.

Thawing iPSC cells

8 Rapidly defrost vial from liquid nitrogen store in 37 °C water bath. Add 4 mL complete E8 media dropwise to defrosted cells in 30 ml white-capped tube and centrifuge at 200 x g for 5 minutes.

9 Aspirate media and resuspend very gently in 1 mL of complete E8 media. Do not triturate more than twice. Transfer to vitronectin coated well and top up with 1 mL complete E8 media. Swirl in figure of eight and check under microscope that you can see clumps of cells.

10 If cell pellet quite large it is best to seed in to two or three 6 wells.

11 Incubate in incubator at 37 °C, 5% CO2. Change media next day. Should see small colonies of cells and very few single cells.

12 Change media daily (2 ml/6 well) until 80-90% confluent.

Passaging iPSC

13 Cells should grow in colonies and with very few differentiated cells. When 80-90% confluent it is time to passage.

14 Wash cells in 6 well with 2 mL room temperature PBS. Aspirate.

15 Add 1 ml of 0.5 mM EDTA in PBS (room temperature) per 6 well (stock EDTA is 500 mM so dilute 1/1000

in PBS; store at room temperature). Incubate at room temperature for 5 minutes.

16 Follow progress of cells under microscope. Colonies should become detached from any differentiating cells at the periphery of the colonies but still remain attached to the plate (if holes begin to appear in the middle of colonies remove EDTA immediately, even if before 5 minutes has elapsed).

17 Carefully aspirate EDTA solution and add 1 ml of complete E8 media. Gently ‘blast’ off colonies with 1 ml pipette. Two or three triturations should be sufficient as you do not want to break up the colonies too much. Remove media containing colonies from well and add to 7 ml of complete E8 media and gently mix with pipette. Plate two ml of cells/vitronectin coated 6 well. This is a 1:4 passage. Do not dilute iPSC more than this.

18 Only passage two or three wells at a time as detached colonies can quickly re-adhere to plates.

19 Change media daily until ready for passage again.