Human pluripotent stem cell culture

FEB 08, 2024

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.j8nlkoq56v5r/v1

Protocol Citation: Jiuchun Zhang, Harper JW 2024. Human pluripotent stem cell culture. **protocols.io** https://dx.doi.org/10.17504/protocols.io.j8nlkoq56v5r/v1

Protocol status: Working

Created: Jan 31, 2024

Last Modified: Feb 08, 2024

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Forked from Human pluripotent stem cell culture

In 1 collection

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ASAP Collaborative Research Network | Harper | 3 more workspaces

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ABSTRACT

This protocol is about human pluripotent stem cell culture.

Some facts about human pluripotent stem cells

1. They attach to several extra cellular matrices, including Matrigel, laminin, vitronectin, fibronectin and high-density RGD peptides. The best and most natural ECM for human PSC is Laminin 511 or 521.
2. Human PSCs don't like to be in single cells. They always grow the best in colonies.
3. The doubling time of human PSC in E8 medium is 16-20 hours.
4. Cells are normally split every 4-5 days.
5. One well of a 6-well plate at 90% confluency will give you roughly 1.5 to 2 million cells.

ATTACHMENTS

Human_pluripotent_stem_cell_culture.pdf

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

PROTOCOL integer ID: 94501

Keywords: hPSCs, Human pluripotent stem cell culture, stem cells, ASAPCRN

Thaw frozen cell from liquid nitrogen tank

1

Safety information

IMPORTANT, ALWAYS WEAR SAFETY GOGGLES WHEN THAWING CELLS!!

2 Take a vial of frozen cells from liquid nitrogen tank. If you have many vials you need to take out of the tank and thaw at a time, you can put them on dry ice while you are looking for other vials.

3 Put the vials in 37 °C water bath with gentle shaking. Do not at any point let water get above 2/3 the height of the vial to prevent water from getting into the vial. **Check the vial periodically.** Take vial out when there is only a small chunk of ice left.

Safety information

This is the step a defective cryovial might explode! So be extra careful and always wear eye protection!

4 Spray the vial with [M] 70 Mass Percent EtOH. Wipe dry. In the hood, open the vial and take the cells out of the vial with a P1000 into a 15 ml conical tube.

5 Slowly add 5 mL to 10 mL fresh E8 to the conical tube with gentle agitation.
Add the first ml drop wise. This is to reduce the osmotic pressure the cells will experience. Do not add thawed cells directly to fresh media!

6 Pellet cells in the conical tube at 150 x g, 00:03:00. Take off supernatant. 3m
Resuspend the cell pellet in appropriate amount of media.

7 Plate the cells onto a matrigel coated plate and put the plate back into the incubator.

8 Cells should attach within an hour of plating.

9 Check cells the next day. Some floating dead cells are OK. The survival is normally 40-70% depending on the freeze.

Regular maintenance of human pluripotent stem cells

10 Feed cells with E8 medium everyday. It is OK to double feed (feed twice the amount of media) and let the cells go without feeding for a couple days only when the cells are not too dense (eg. Right after a split).

11 When cells are 70-90% confluent, they need to be split.

12 To split cells, follow the steps below. The volume is for one well of a 6-well plate. Change the volume of solution according to your culture vessel.

13 Take off media and rinse with 1 mL PBS+EDTA (0.5 mM EDTA final concentration). Take off the 5m
PBS+EDTA and add another 1 mL PBS+EDTA. Let the plate sit in the hood for 00:03:00 -

00:05:00.

14 Look under the microscope. The cells within a colony should start to separate from each other. If they are still attached to each other, wait for another 00:02:00 - 00:03:00 and check again. 3m

15 Carefully take the PBS+EDTA off the plate. If the cells are incubated too long with EDTA, they might come off the plate at this step. So be careful not to suck up your cells.

16 Resuspend cells with 3 mL fresh E8 using a 5 ml pipette. Dislodge the cells only with media. DO NOT scrape cells off the plate with pipettes. Pipette cells up and down several times to break up cell clumps.

17 Aliquot resuspended cells into another plate with fresh E8.

18 I normally split cells at series of different ratios (1:6, 1:12 and 1:20).

Note

A 1:6 split will normally give you 50-70% confluency after 2 days. This is good for electroporation. A 1:20 split will give you 70-90% confluency after 4-5 days and this is good for regular maintenance especially for double feeding over the weekend.

Freezing cells

19 Split cells at 1:3 or 1:6 two days before freeze. Cells 2 days after split will give the best survival after thaw. You can still freeze cells 4 or 5 days after split if you have to. But the survival will be significantly reduced especially if the cells are confluent.

20 Label appropriate number of cryovials you need for freezing. Also make sure you have appropriate number of Mr. Frosty.

21 Treat cells with EDTA just like what you would do when you split cells.
Resuspend cells with fresh E8 in half the volume you want to freeze your cells in.

22 Prepare [M] 20 Mass Percent DMSO in E8 by mixing 8 parts of E8 with 2 parts of DMSO.

23 Slowly add the same volume of [M] 20 Mass Percent DMSO in E8 to the resuspended cells with gentle mixing. This will give [M] 10 Mass Percent DMSO final concentration in the freezing solution.

24 Aliquot the cells into appropriate number of cryovials.

25 Put vials into Mr. Frosty and place the Mr. Frosty in a -80 °C freezer.

26 The next day, transfer all the vials to liquid nitrogen tank for long-term storage. Human pluripotent stem cells are good for several months at -80 °C. But it is general not recommended to keep your frozen cells at -80 °C for an extended period of time.

27 (Optional). Thaw one vial of cells to check on the survival rate. Repeat the freezing if survival is not optimal.

28 Generally, a 70-80% confluent well in a 6-well plate can give you 2-3 vials cells.