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Human Pluripotent Stem Cell Culture

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We use this protocol and it's working

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Abstract

This protocol described how to perform routine maintenance of human induced pluripotent stem cells (iPSCs), including freezing and thawing.

Coating Plates with Laminin-521

1 Coat your plates with Laminin-521 (0.7 µg/cm²; Biolamina) diluted in DPBS +/+ at 37°C for at least 2 hours before use or at 4°C overnight.

Thaw Frozen Cells

2 Take your vials of frozen cells and keep them on dry ice until ready.

3 Prepare one 15 ml tube with wash buffer (9.5 ml DMEM/F-12 + 0.5 ml KSR) per vial.

4 Put the vials in 37°C water bath. Check the vial periodically. Take vial out when there is only a small chunk of ice left.

5 Spray the vial with 70% EtOH. Wipe dry. In the hood, open the vial and take the cells out of the vial with a P1000 into your tubes with wash media.

6 Pellet cells in the conical tube at 400g for 5 min. Take off supernatant. Resuspend the cell pellet in appropriate amount of iPS media supplemented with Rock inhibitor (StemMACS iPS-Brew XF, 0.5% penicillin/streptomycin and 10 µM of Y27632).

7 Plate the cells onto a Laminin-521 coated plate and put the plate back into the incubator.

Regular Culture of Human Pluripotent Stem Cells

8 Feed cells with pre-warmed iPS media every day (StemMACS iPS-Brew XF with 0.5% penicillin/streptomycin).

9 Split the cells when they are 70-90% confluent.

9.1 Take off media and rinse with 1 ml PBS -/-.

9.2 Add 500 µl of Accutase and incubate at 37°C for 7-10 minutes.

9.3 Monitor cell detaching under the microscope.

9.4 Harvest your cell suspension into a 15 ml tube with pre-warmed wash buffer.

9.5 Pellet cells in the conical tube at 400g for 5 min. Take off supernatant. Resuspend the cell pellet in appropriate amount of iPS media supplemented with Rock inhibitor (StemMACS iPS-Brew XF, 0.5% penicillin/streptomycin and 10 µM of Y27632).

9.6 Put the new plates in the incubator.

Freezing Cells

10 Split cells following the same steps as in the section above.

11 Count your cells with a cell counter and transfer the desired amount to a new 15 ml tube (we normally freeze 300,000 cells per vial) and spin at 400xg for 5 min.

12 Resuspend the pellet in the appropriate volume of Cryostor (1 ml per vial) and immediately transfer to the labeled cryotubes.

13 Put vials into Mr. Frosty and place the Mr. Frosty in a -80°C freezer as quickly as possible.

14 The next day, transfer all the vials to -150°C freezer or liquid nitrogen for long-term storage.