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Cultivation of hiPS cells on Laminin-521 (LN521)

iPS Core
National human iPS cell core facility
Karolinska Institutet
www.iPScore.se

Introduction

Cells grewing on Laminin521-coated plastic are passaged when they have reached about 80% confluency. Medium is changed every day. This protocol covers cultivation in **12-well plates**, for any other size/format, the volumes of reagents and number of cells for seeding need to be adjusted.

Materials

E8 medium (with supplement) Gibco # A1517001, LifeTechnologies

PBS without calcium and magnesium (Ca^{2+} and Mg^{2+}), Gibco #14190-94 LifeTechnologies

TrypLE Select, Gibco # 12563-011 LifeTechnologies

Penecillin/Streptomycin (PE/ST), Gibco # 15140-122 LifeTechnologies

[OPTIONAL only] Antibiotic-Antimycotic/ Fungizone (Amphotericin B)

ROCK inhibitor (Y-27632) #SCM075 Millipore

12-well platesSarstedt (3921.300) or VWR (734-2324)

Laminin-521, BioLamina

TrypanBlue + Burker chamber or any other counting device

Parafilm

Methods

Preparing Plates and Medium

Coating Plates

- Thaw Laminin-521, thawed solution is stable for 3 months at +4°C
- Laminin-521 (100 µg/ml) is diluted x20 in PBS. Use freshly diluted Laminin for coating.
- Add diluted LN521 (5µg/ml) 500 µl per well

- Seal with Parafilm and leave at +4°C overnight, plates can be stored for up to 2 weeks at +4 °C.
- Alternatively, coat with the same amount of Laminin and incubate for at least 2 hours at +37°C.

Medium

- Thaw Essential 8 medium Supplement overnight at +4 °C. Do not thaw at +37°C.
- Mix 490 ml of medium with 10 ml of Supplement and add 5 ml PE/ST to the medium. The medium can be stored at +4°C for up to two weeks. Before use, warm the medium required for the day to room temperature. Do not warm at +37°C.
- Alternatively, and recommended, thaw the Supplement as mentioned and freeze it in 1 ml aliquots, after thawing at room temperature mix with 49 ml of medium and add 500 µl PE/ST.

Chemicals

- ROCK Inhibitor (Y-27632), 338 g/mol. Reconstitute the powder, 5 mg in 3 ml of sterile water to a concentration of 5 mM.
- Aliquot and store at -20°C. Dilute x500 to a final concentration of 10 µM.

Passaging the cells

- Let the TrypLE Select and E8 medium warm up to room temperature before you start.
- The following amounts are for one well of 12-wellplate, adjust volumes accordingly.
- Aspirate the medium from the cells and wash with 1 ml PBS (no Ca2+, no Mg2+).
- Add 500 µl of TrypLE Select and incubate for 3 minutes at +37C.
- Look at the cells, they should have loosened up a bit, make sure the cells come off the plate by pipetting up and down the TrypLE Select solution for a few times.
- Add 1 ml of E8 medium to a tube, transfer the cells to the tube and centrifuge for 3 minutes at 1300 rpm.
- Aspirate the medium and resuspend the cells in 1 ml of fresh medium, then count them.
- Aspirate the Laminin from the prepared wells. Do not wash the wells.
- seed 70 000-100 000 cells/well. (17 500 – 25 000 cells/cm^2)

- Add ROCK Inhibitor (Y-27632) to a final concentration of 10 µM. Observe that ROCK is only added after passaging cells, not when feeding daily.
- Feed the cells daily (change medium every day) and then passage or freeze them when they reached about 80% confluency.