Cultivation and passaging of hiPS cells on Laminin-521

Cells grow on Laminin coated plastic and 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 the volumes need to be adjusted.

Materials

E8 medium (with supplement) # A1517001, Gibco

PBS without Ca and Mg, Gibco

TrypleSelect # A1217701, Gibco

PenStrep, Gibco

ROCK inhibitor (Y-27632)

12-well plates, Costar, NUNC or Sarstedth

Laminin-521, BioLamina

TrypanBlue + Burker chamber or any other counting device

Parafilm

Methods

Preparing Plates and Medium

Plates

Thaw Laminin-521 overnight at +4^{o}C, thawed solution is stable for 3 months at +4^{o}C

Laminin-521 (100 µg/ml) is diluted x10 in PBS. Use freshly diluted Laminin for coating.

Add 500 µl/well (1.25 µg/cm^{2}).

Seal with Parafilm and leave at +4^{o}C overnight, plates can be stored for up to 4 weeks at +4 ^{o}C.

Alternatively, coat with the same amount of Laminin and incubate for 2 hours at +37^{o}C.

Medium

Thaw Essential 8 medium Supplement overnight at +4 ^{o}C. Do not thaw at +37^{o}C.

Mix 490 ml of medium with 10 ml of Supplement. 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 and add 10 µl PenStrep/ml medium. Do not warm at +37°C.

Alternatively, 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 PenStrep.

Passaging the cells

Let the TrypleSelect and E8 medium warm to room temperature before you start

Aspirate the Laminin from the prepared wells and replace with E8 medium. Leave at room temperature.

The following amounts are for a 12well plate, adjust volumes accordingly;

Aspirate the medium from the cells and wash with 1 ml PBS.

Add 500 µl of TrypleSelect 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 for a few times.

Add 1 ml of E8 medium and centrifuge for 3 minutes at 1400 rpm.

Aspirate the medium and resuspend the cells in 1 ml of medium.

Count the cells and seed 200 000 cells/well.

Add ROCK Inhibitor (Y-27632) to a final concentration of 10 µM.

Change ¾ of the medium every day.

Cells are usually ready to passage after 4-6 days.