



iPS cell generation procedure (For research use only)

Note: Human iPS cells were successfully generated from human patient fibroblast cells in 14 days using Gentarget Inc's human iPS lentivirus with this protocol. However, since each cell type is different, this protocol was for your reference only.

Day 0: Seed the parent cells:

For example, seed human fibroblast cells at 1×10^5 cells/well in 6-well plate, cultured in 5ml of growth medium, incubated overnight at 37°C with 5% CO₂.

Day 1: Viral Transduction:

On the second day, remove medium, add 1ml of pre-warmed fibroblast growth medium, then add 50 ul of each iPS lentivirus (Oct3/4, Sox2, NANOG, LIN28, c-Myc and Klf4, total 300ul of lentivirus) [Note: you may not need to use all iPS factors dependent upon your cell types. But reported showed this full set was successfully induced mouse adult fibroblast into iPSC cells]. Gentle mix for evenly distribution, then incubated overnight at 37°C with 5% CO₂.

Day 2: Optional: another transduction:

For some cell line, remove medium, and 1 ml of pre-warmed fibroblast growth medium, then add 50 ul of each iPS lentivirus for another transduction, incubated overnight at 37°C with 5% CO₂.

Day 3: Change Medium:

At about 48 hours after the 1st transduction, change medium with 5 ml fibroblast growth medium.

Day 5: Re-plate the transduced cells to feeder cells

At four Days of post-transduction, the cells were trypsinized, centrifuged at 200 x g for 5 minutes, resuspended in Fibroblasts Cell Growth Medium, and re-plated in a 150mm MEF Feeder Dishes. These cells were incubated overnight at 37°C with 5% CO₂.

Day 6: Change medium to reprogramming medium:

At 24 hours after re-seeding, Fibroblast Cell Growth Medium was replaced with Human ES/iPS Cell Medium (HiPS Cells Food). The medium was changed every day for the first 7 days. During this period, all ES cell-like colonies were selected and re-seeded in Human ES/iPS



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Cell Medium supplemented with 10 μ M Stemolecule™ Y27632 on CF-1 MEF Plates. Human ES/iPS Cell Medium was changed every day.

Day 13: Change to Condition Medium:

After re-programming in ES/iPS medium for 7 days, change medium into MEF Conditioned Medium. Continue to pass the cells until they showed typical human ES cell morphology.

Attachment: Reprogramming Medium:

HiPS food plus VPA, PD, SB and Tvz

**iPS cells generated from human patient fibroblast cells by using
Gentarget Inc's lentivirus set (Cat#: [LVP-stems-h](#))**

