Human iPS Cell Reprogramming Retrovirus Kit

Catalog Number RF101

Description

Human induced pluripotent stem cells (iPSCs) can be derived from somatic cells through a reprogramming process driven by ectopic expression of a defined set of reprogramming factors: Oct4, Sox2, Klf4, and c-Myc. These hiPSCs share the properties of self-renewal and pluripotency with human ES cells, and can, therefore, be used as a renewable source for all differentiated cell types of the body. Human iPSCs can be generated from patients of virtually any genetic background.

Retroviruses are efficient tools for delivering heritable genes into the genome of dividing cells. The VSV-G pseudotyped retrovirus has a wide range of targets, including both mammalian and non-mammalian cells, and are usually silenced in ES cells. The retrovirus is commonly used in generating iPSCs because of its high reprogramming efficiency. The Human iPS Cell Reprogramming Retrovirus Kit offers such an opportunity to generate iPSCs from various tissues and cell types.

Contents	Retrovirus cocktail containing: human Oct4 retrovirus (Cat. # RF01O) - 2 vials, 20 µl each human Sox2 retrovirus (Cat. # RF01S) - 2 vials, 20 µl each human Klf4 retrovirus (Cat. # RF01K) - 2 vials, 20 µl each human c-Myc retrovirus (Cat. # RF01M) - 2 vials, 20 µl each GFP retrovirus (Cat. # RF01G) - 1 vial, 20 µl each TransPlus virus transduction enhancer (Cat. # V020) - 1 vial, 100 µl
Storage and Stability	Store at -80° C. This product is stable up to 6 months when stored as directed.
Quality Control	Each lot of Human iPS Cell Reprogramming Retrovirus Kit is tested for sterility, and verified the ability to convert fibroblasts to iPS cells.
Restricted Use	For Research Use Only. Not for use in diagnostic or therapeutic procedures.

Protocol

Retroviral transduction of human dermal fibroblasts

Material needed:

Human ES Medium:

DMEM/F12	Invitrogen	10565-042	1X	
Pen/Strep	Invitrogen	15140-122	1X	1 ml
L-Glutamax	Invitrogen	35050-061	2 mM	2 ml
Nonessential Amino Acids	Invitrogen	11140-050	0.1 mM	2 ml
2-mercaptoethanol	Sigma	M7522	0.1 mM (1000X)	0.2 ml
Knockout Serum Replacement	Invitrogen	10828-028	20%	40 ml
bFGF	StemRD	bFGF-050	10 ng/ml	0.2 ml
			Total	200ml

Procedure:

1. When human Fibroblasts reach 80% confluence, aspirate medium, wash twice with PBS, cover cells with 0.05% trypsin, and incubate for 5 min at 37°C.

2. Inactivate trypsin with fresh culture medium, and collect cells into a 15 ml conical tube.

3. Centrifuge cells at 200x g at room temperature for 5 min and discard the supernatant.

4. Resuspend the cells in 1 ml fresh culture medium and count the cell number using a hemocytometer.

5. Plate 1 x 10^5 cells in each well of 6-well plate, and incubate cells at 37°C, 5% CO_2, for 6 hours.

6. Aspirate medium to remove dead cells, and add 2 ml of fresh culture medium.

7. Add retroviruses carrying hOCT4, hSOX2, hKLF4, and hc-MYC, respectively. Infect one well with retroviruses at MOI 10 and one well with the retrovirus carrying GFP and one with empty vector as control.

8. Add 4 µl of 500x TransPlus (cat# V020, ALSTEM) solution into each well, and mix gently by swirling the plate.

9. Repeat steps 7 and 8 the next day.

10. One day after the final infection, remove the viral supernatant, wash three times with PBS, and add 3 ml of fresh culture medium.
11. Four days after infection, plate 2 x 10^6 mitomycin C treated MEF cells in a 100-mm dish or two 60-mm dishes (precoated with 0.1% gelatin, cat. no. M500, ALSTEM). Incubate until the next day.

12. On day 5 after the first infection, trypsinize the infected cells and plate them in a 100-mm dish at different cell densities between 5 x 10^4 to 2 x 10^5 cells or in a 60-mm dish at densities between 2 x 10^4 to 1 x 10^5 cells.

13. Two days later, aspirate medium and replace it with hES medium.

14. Change medium every day with hES medium.

15. After about 3-4 weeks, check the colony formation and pick the with ES-like morphology manually for expansion in hES media.

Progress of reprogramming human fibroblasts