Applied StemCell, Inc.
(866) 497-4180
www.appliedstemcell.com

Datasheet

EZ-iPSC Generation kit – RETROVIRAL

Product Information

Catalog Number

ASK-3012 (Retroviral VSV-G based iPSC reprogramming kit)

Description

Human induced pluripotent stem cells (hiPSCs) 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 EZ-iPSC Generation Retrovirus Kit offers such opportunity to generate iPSCs from various tissues and cell types with an increased reprogramming efficiency of 0.05-0.5%. The success of iPSC generation and subsequent differentiation relies on the efficient and rapid silencing of exogenous genes. The exogenous genes delivered by retroviruses can be silenced faster than those from lentiviral-based vectors. This makes the retroviral kit a powerful and versatile tool for generating iPSCs from various tissues or cell types.

Shipping

Dry ice

Storage and Stability

Store individual components provided in the kit as directed in Media and Material table. This product is stable for up to 6 months when stored as directed.

Quality Control

Each lot of EZ-iPSC Generation Retrovirus Kit is tested for sterility, and verified for the ability to convert fibroblasts to iPS cells. The amount of each virus has been carefully optimized to reach its highest reprogramming efficiency. Every lot of the cocktail is tested for reprogramming human foreskin fibroblasts.

Safety Precaution

PLEASE READ BEFORE HANDLING ANY FROZEN VIALS. Please wear the appropriate Personal Protection Equipment (lab coat, thermal gloves, safety goggles and a face shield) when handling the cells. Handle the frozen vials with due caution. Please be aware that the following scenario can occur: Liquid nitrogen can leak into the vials when the vials are submerged in liquid nitrogen. Upon thawing, the liquid nitrogen returns to the gas phase, resulting in a dangerous build-up of pressure within the vial. This can result in the vial exploding and expelling not only the vial contents but also the vial cap and plastic fragments of the vial.

Restricted Use

Reagents and materials included in EZ-iPSC Generation Kit are intended for research purposes only. It is not for use in diagnostic or therapeutic procedures.

Applied StemCell, Inc.
521 Cottonwood Dr. #111, Milpitas, CA 95035
Phone: 866-497-4180 (US Toll Free); 408-773-8007 Fax: 408-773-8238
info@appliedstemcell.com www.appliedstemcell.com
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Media and Material

Catalog #	Component	Product details	Storage
ASR-2005-a	VSV-G OCT4 Retrovirus	20 µL/ vial; 2 vials	-80ºC
ASR-2005-b	VSV-G SOX2 Retrovirus	20 µL/ vial; 2 vials	-80ºC
ASR-2005-c	VSV-G KLF4 Retrovirus	20 µL/ vial; 2 vials	-80ºC
ASR-2005-d	VSV-G hc-MYC Retrovirus	20 µL/ vial; 2 vials	-80ºC
ASR-2006	GFP VSV-G Retrovirus	20 µL/ vial; 1 vial	-80ºC
ASM-5002	DMEM/F12	400 mL	4˚C
ASF-1217	CF1 MEF, Irradiated	1 vial	Liquid N_2
ASK-3501-2	Virus Transduction Enhancer	100 µL	4°C

Materials and Instruments Required but not Provided

Component	Vendor	Catalog #	Conc.	Volume
Penicillin/streptomycin	ThermoFisher	15140122	1X	1 mL
L-Glutamax	ThermoFisher	35050061	2mM	2 mL
Non-essential amino acid	ThermoFisher	11140050	0.1mM	2 mL
2-mercaptoethanol	Sigma	M7522	0.1 mM (1000x)	0.2 mL
Knockout serum replacement	ThermoFisher	10828028	20%	40 mL
bFGF	Stem RD	bFGF-050	10 ng/mL	0.2 mL
Coating Matrix/ Gelatin				

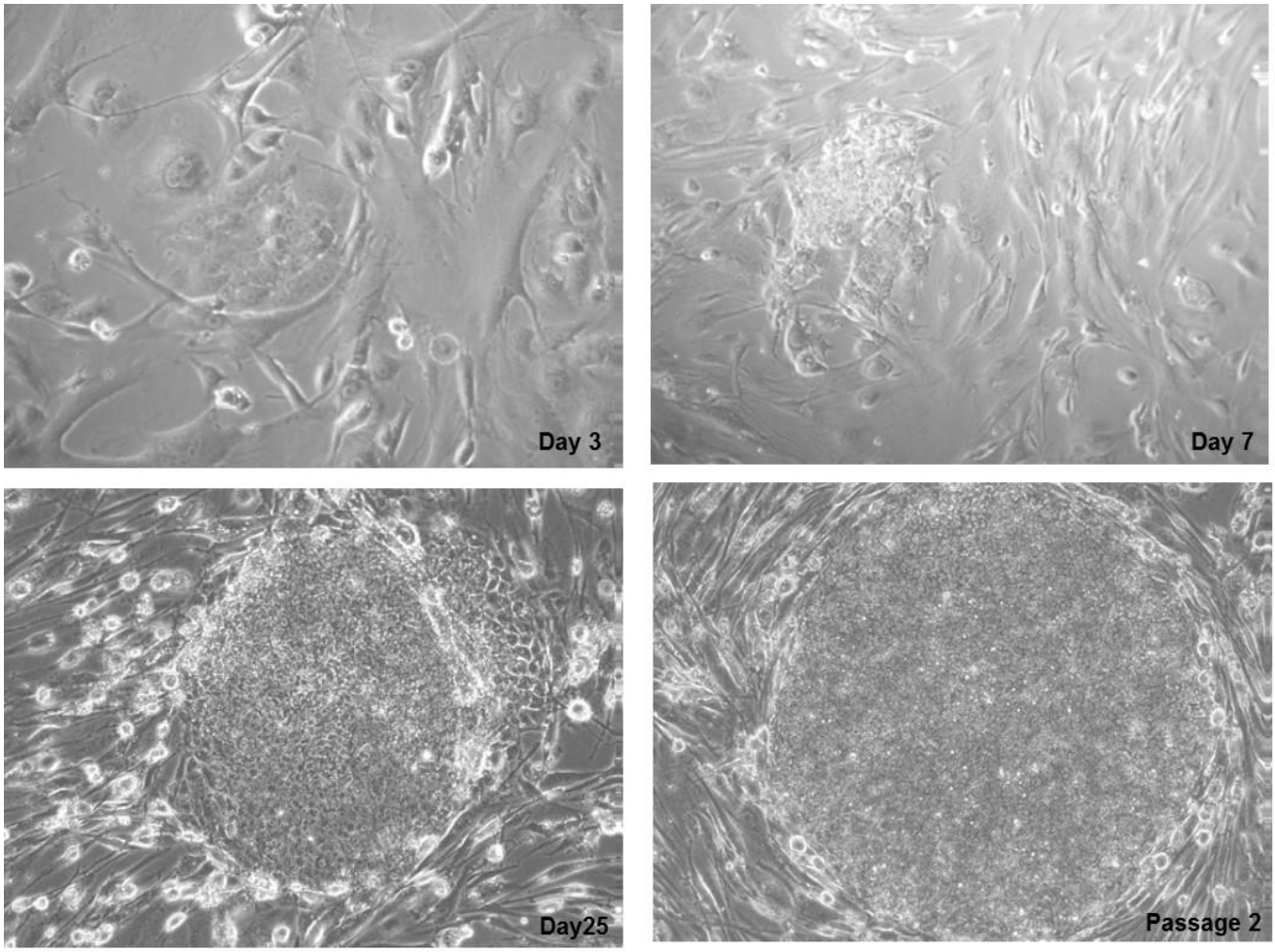
Protocol

Retroviral transduction of human dermal fibroblasts

1. When human fibroblasts reach 80% confluence, aspirate medium, wash twice with PBS, cover cells with 0.05% trypsin, and incubate for 5 minutes 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 minutes 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 5 - 10 µL of concentrated retroviral particles for each unique retrovirus carrying hOCT4, hSOX2, hKLF4 and hc-MYC, respectively, to a single well of 6-well plate. Infect one well with retrovirus carrying GFP as control.
8. Add 4 µL of 500x Virus Transduction Enhancer solution into each well, and mix gently by swirling the plate.
9. Repeat steps 7 and 8 next day.
10. One day after 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 (pre-coated with 0.1% gelatin). Incubate until the next day.
12. On day 5 after 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 with hES medium.
14. Change medium everyday 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



References

1. Takahashi, K., Yamanaka, S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. Cell. 2006. 126: 663-76.

2. Takahashi, K., et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell. 2007. 131: 861-72.

3. Miura, K., et al. Variation in the safety of induced pluripotent stem cell lines. Nat Biotechnol. 2009. 27: 743-5.