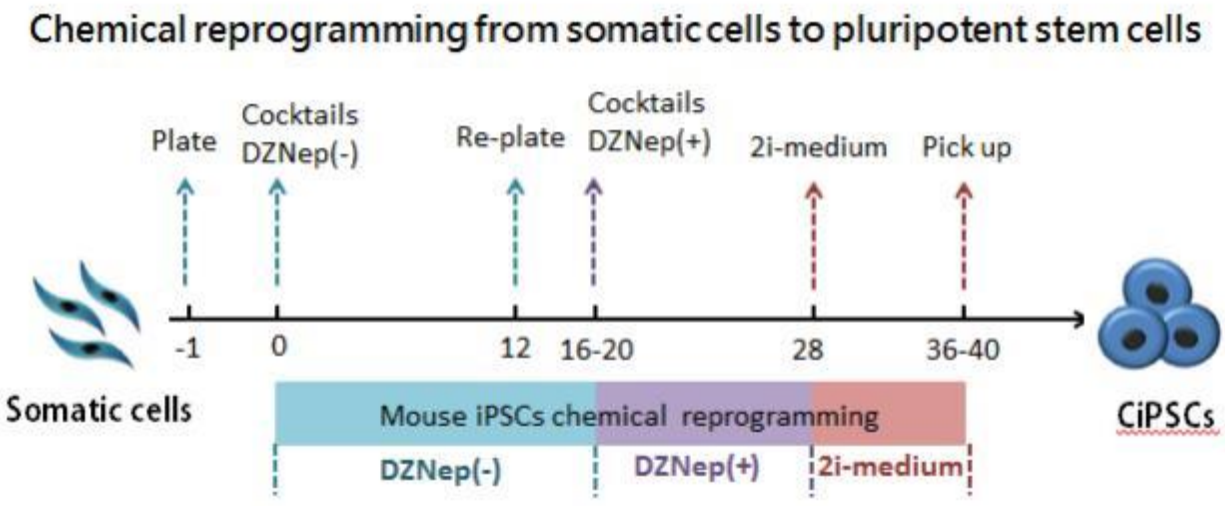
Mouse iPSC Chemical Reprogramming Cocktails Kit

Product Description

The cocktails composed of 7 small molecule compounds (VC6TFZ+TTNPB) can be used to generate pluripotent stem cells from mouse somatic cells (such as MEFs, MNFs, MAFs or ADSCs) at a frequency of up to 0.2%.This chemical reprogramming technology has potential application value in generating functional cell types required for clinical applications[1]. Multipotential stem cells are self-replicating cells that can be induced from somatic cells through nuclear transfer to oocytes, transgene delivery, or treatment with compounds, and then differentiate into three primary germ layers[1]. To achieve complete chemical reprogramming without the Oct4 inducible system, these small molecules were further tested in chemical reprogramming of OG-MEFs without the use of transgenes. When DZNep was added after 16 days of treatment with VC6TF (VC6TFZ), a higher frequency of GFP-positive cells was obtained. The expression levels of most pluripotency marker genes increased but were still lower than in ESCs. After switching to 2i-medium containing dual inhibition (2i): CHIR-99021 (CT99021) and PD0325901 with glycogen synthase kinase-3 and mitogen-activated protein kinase signals, some GFP-positive colonies formed developed an ESC-like morphology, namely chemically induced pluripotent stem cells (CiPSCs).Another screen identified a synthetic retinoic acid receptor ligand, TTNPB, which increased chemical reprogramming efficiency to a level comparable to that of transcription factor-induced reprogramming (up to 0.2%)[1]. In immunodeficient (SCID) mice injected with CiPSCs, the cells were able to differentiate into tissues of the three germ layers. When CiPSCs are injected into eight-cell embryos or blastocysts, CiPSCs are able to integrate into organs of all three germ layers, including gonads, and are passed on to offspring.Chimeric mice generated from CiPSCs have a 100% survival rate and remain healthy for up to 6 months, indicating that fully reprogrammed CiPSCs are pluripotent[1].



Product components and storage conditions

The kit contains a Chemical Reprogramming Cocktail and Dual Inhibition (2i) Medium Additive as shown in the table below:

Chemical Reprogramming Cocktail

Cat No	Compound Name	Target	Cocktail	Size (for 100 ml medium)	Size (for 500 ml medium)
A4099	Valproic acid sodium salt (Sodium valproate)	HDAC inhibitor	0.5 mM	10 mg	50 mg
A3011	CHIR-99021 (CT99021)	GSK-3 inhibitor	10-20 μM	1 mg	5 mg
A3754	RepSox (616452)	ALK5 inhibitor	5-10 μM	1 mg	2 mg
B7514	Tranylcypromine hydrochloride	LSD1/MAO inhibitor	5-10 μM	1 mg	1 mg
B1421	Forskolin	Adenylate cyclase activator	10-50 μM	2.5 mg	12.5 mg
B2058	TTNPB (Arotinoid Acid)	RAR agonist	1 μM	1 mg	1 mg
A8182	3-Deazaneplanocin A	SAH and ENZ2 inhibitor	0.05-0.1 μM	1 mg	1 mg

Dual Inhibition (2i) Medium Additive

Cat No	Compound Name	Target	Final Concentration	Size (for 500 ml medium)
A3011	CHIR-99021 (CT99021)	GSK-3 inhibitor	3 μM	1 mg
A3013	PD0325901	MEK inhibitor	1 μM	1 mg

- Store at 20°C and ship with blue ice

***Note:**

When MEFs are induced to generate CiPSCs, the recommended concentration of CHIR-99021 is 10 μM. When MNFs, MAFs, ADSCs or no replating operation is used to induce the generation of CiPSCs, the recommended concentration of CHIR-99021 is 20 μM (day 0-12).

When MEFs are induced to generate CiPSCs, Forskolin recommends using a concentration of 10 μM. When MNFs, MAFs or ADSCs are induced to generate CiPSCs, Forskolin recommends using a concentration of 50 μM (day 0-12).

Operation

Stages	Time	Process
Plate	Day -1	Cell seeding: Plate primary cells (MEFs, MNFs, MAFs or ADSCs) at 300,000 cells in a 10 cm dish or 50,000 cells per well in a 6-well plate.
Stage 1	Day 0	The culture medium was changed to medium containing small molecule cocktails, and the medium containing small molecule cocktails was changed every 4 days.

Re-plate	Day 12-16	On day 12, cells were washed with PBS, and digested with 0.25% trypsin-EDTA at 37℃ for 3-5 minutes. Neutralize and dissociate cell clumps into individual cells. Cells are harvested (300,000-1,000,000 cells per well in a 6-well plate), resuspended, and replated into chemical reprogramming medium containing small molecule cocktails at a density of 300,000-500,000 cells per well in a 6-well plate.
Stage 2	Day 16 or 20	Add DZNep to the cell culture medium on day 16 or 20.
Stage 3	Day 28	On day 28, small molecule cocktails including DZNep were removed. At the same time, replace the chemical reprogramming medium with 2i-medium (ESC medium supplemented with 2i (3 μM CHIR99021 and 1 μM PD0325901).
Pick up	Day 36-40	Count 2i-competent, ESC-like and GFP-positive colonies as primary CiPSC colonies. When CiPSCs are induced from wild-type cells without the OG reporter gene, 2i-competent and ESC-like colonies are counted as primary CiPSC colonies. These CiPSC colonies were selected for amplification and characterization. An alternative approach is to induce CiPSCs on day 12 without replication.

Reference

1. Hou P, Li Y, Zhang X, Liu C, et al. Pluripotent stem cells induced from mouse somatic cells by small-molecule compounds. Science. 2013 Aug 9; 341(6146):651-4.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com