Mouse iPSC Chemical Reprogramming Cocktails Kit plus

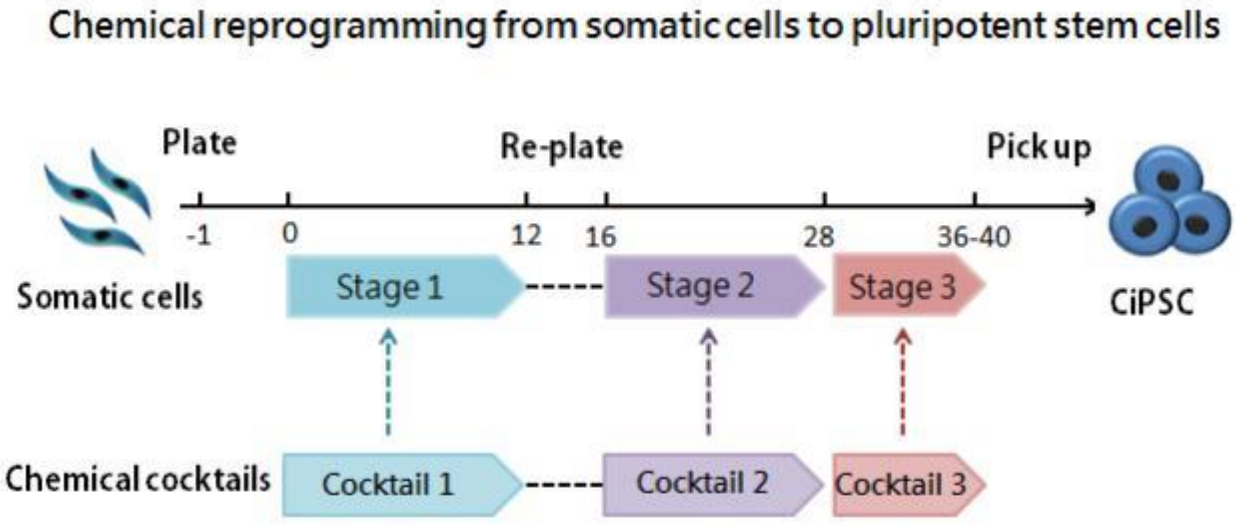
Description

"VC6TF + EPZ004777 + AM580", "VC6TFZ + AM580 + SGC0946 + 5-aza-dC" and "N2B27-2i + LIF" are three novel small molecule cocktails that enhance the chemical reprogramming efficiency of newly characterized extraembryonic endoderm (XEN)-like cells (i.e., somatic to embryonic stem cells)[1]. "

Multipotential stem cells are self-replicating cells that can be generated from somatic cells induced by nuclear transfer to oocytes, transgene delivery, or treatment with compounds, and then differentiated into three primary germ layers[1].

The chemical reprogramming process consists of three essential stages. In the first stage, the RA agonist AM580 (A) and the DOT1L inhibitor EPZ004777 (E) increase the formation of XEN-like colonies by 2-3-fold. The number of XEN-like colonies was increased more than 5-fold in cocktails consisting of seven small molecules (VC6TFAE: VPA, CHIR99021, 616452, tranylcypromine, forskolin, AM580 and EPZ004777). Replacing EPZ004777 with another DOT1L inhibitor, SGC0946 (S), in the second stage further increased the reprogramming efficiency by up to 5-fold, especially when using the optimized 2i-medium (N2B27-2iL medium). In addition, CiPSC (chemically induced pluripotent cells) colonies were generated in Phase III only when supplemented with 5-aza-Dc (D) in Phase II. Using the small molecule Cocktails VC6TFAZDS in the second phase for 12 days, 100 - 600 CiPSC colonies can be induced from 50,000 re-plated cells in the final phase of chemical reprogramming[1].

All six CiPSC colonies examined could form teratomas after injection into SCID mice and could produce chimeric mice after blastocyst injection. Four CiPSC colonies showed potential for germline integration in chimeric mice, and germline transmission progeny were obtained from chimeric mice[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 1/2

Cat No	Compound Name	Target	Cocktail 1	Cocktail 2	Size (for 100 ml medium)	Size (for 500 ml medium)
A4099	Valproic acid sodium salt (Sodium valproate)	HDAC inhibitor	0.5 mM	0.5 mM	10 mg	50 mg
A3011	CHIR-99021 (CT99021)	GSK-3 inhibitor	20 μM	10 μM	1 mg	5 mg
A3754	RepSox (616452)	ALK5 inhibitor	10 μM	10 μM	1 mg	2 mg
B7514	Tranylcypromine hydrochloride	LSD1/MAO inhibitor	5 μM	5 μM	1 mg	1 mg
B1421	Forskolin	Adenylate cyclase activator	50 μM	10 μM	2.5 mg	12.5 mg
B4654	AM580	RARα agonist	0.05 μM	0.05 μM	1 mg	1 mg
A4170	EPZ004777	DOT1L inhibitor	5 μM	N/A	1 mg	2 mg
A8182	3-Deazaneplanocin A	SAH and ENZ2 inhibitor	N/A	0.05 μM	1 mg	1 mg
A1906	Decitabine (NSC127716, 5AZA-CdR)	Cellular differentiation inducer	N/A	0.5 μM	1 mg	1 mg
A4167	SGC 0946	DOT1L inhibitor	N/A	5 μM	1 mg	2 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

Operation

Stages	Time	Process
Plate	Day -1	MEF cell inoculation: MEF was inoculated at 300,000 cells in 10 cm dishes or 50,000 cells per well in 6-well plates.
Stage 1	Day 0	Change culture solution to Phase I medium（include 100 ng/ml bFGF, 0.5 mM VPA, 20 μM CHIR99021, 10 μM 616452, 5 μMtranylcypromine, 50 μM forskolin, 0.05 μMAM580 and 5 μM EPZ004777）
Re-plate	Day 12-16	On day 12, cells were digested with trypsin, harvested, resuspended, and repopulated in 6-well plates at 50,000-200,000 cells/well (1:10-15). During days 12-16, the concentrations of bFGF, CHIR and forskolin were reduced to 25 ng/ml, 10 μM and 10 μM, respectively.
Stage 2	Day 16	XEN-like epithelial colonies were formed and the culture medium was changed to phase II medium(containing 25ng/ml Bfgf, 0.5mM VPA, 10μM CHIR99021, 10μM 616452, 5 μMtranylcypromine, 10μM forskolin, 0.05Mm AM580, 0.05μM DZNep, 0.5 μM 5-aza-dC and5 μM SGC0946).
Stage 3	Day 28	The culture was transferred to Phase III medium (with 3 μM CHIR99021, 1 μM PD0325901 and 1000 U/ml LIF N2B27-2iL medium).
Pick up	Day 36-40	After 8-12 days, 2i-competent, ESC-like, and GFP-positive CiPSC colonies appeared (if applied with pOct4-GFP reporter gene), the amplification and characterization were then performed.

Reference

1. Zhao Y, Zhao T, Guan J et al. A XEN-like State Bridges Somatic Cells to Pluripotency during Chemical Reprogramming. Cell. 2015 Dec 17; 163(7):1678-91.

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