Data Sheet

STEMCCA™ Lentivirus Reprogramming Kits

Efficient iPS Cell Generation with a Single Vector

A ready source of induced pluripotent stem cells (iPS cells) is critical to the effective study of differentiation pathways or the investigation of the therapeutic potential of iPS cells.

More convenient and reliable than embryonic stem cells, which are often more heterogeneous, hard to culture and subject to regulation, iPS cells offer a significant opportunity to not only propel, but dramatically enhance the efficiency and effectiveness of stem cell research overall.

Merck Millipore STEMCCA lentivirus reprogramming kits make it easier than ever to obtain and work with induced iPS cells, addressing the key challenges facing iPS cell generation.

STEMCCA Vector Advantages:

- Efficient: uses a single vector with four transcription factors rather than co-transducing four separate expression vectors
- Minimizes viral integrations: single vector reduces the risks of insertional mutagenesis and viral reactivation
- Excisable: Cre/LoxP-regulated version enables removal of reprogramming transgenes

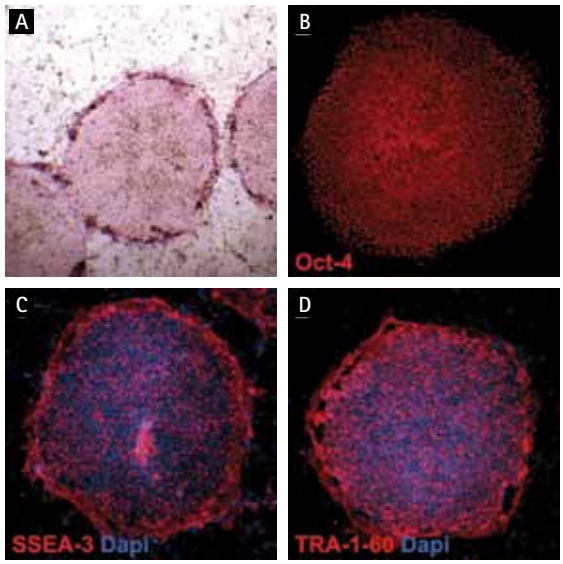


Figure 1. Successful generation of iPS cells from human foreskin fibroblasts after infection with single-vector Human STEMCCA Cre-Excisable Lentivirus (Cat. No. SCR545), as indicated by expression of characteristic pluripotency markers. Resulting passage 3 human iPS cells exhibit high alkaline phosphatase activity (A), Oct-4 expression (B), SSEA-3 expression (C), and TRA-1-60 expression (D). Nuclei are stained with DAPI (blue).

The Challenge: Efficiently Generating Reproducible iPS Cells

Traditionally, adult cells are reprogrammed through the co-infection of the four Yamanaka transcription factors (Oct-4, Klf4, SOX-2, and c-Myc (OKSM)) in four separate expression vectors[3-7]. For successful reprogramming, a sufficient number of each virus must deliver the four factors simultaneously to the same cell, raising concerns over the high number of integration sites and the difficulty in removing these viral integrations from genomic DNA. Moreover, the inability to predict whether cells receive one, two, three, or all four factors has created heterogeneous cell populations, further complicating detailed study into the mechanism and timing of reprogramming.

The Solution: Single-Vector Reprogramming with STEMCCA Technology

Unlike traditional iPS generation, which requires simultaneous co-infection by four separate expression vectors, the STEMCCA kits use a single polycistronic lentiviral vector to improve efficiency and reduce the number of viral integrations. It also enables the creation of more homogeneous, reproducible iPS cell populations—in some cases, iPS clones which possessed only a single viral integrant were isolated[1].This polycistronic cassette technology has also been applied toward generating single-gene transgenic mouse strains[8].

Furthermore, removing the reprogramming vector (an option with the STEMCCA Cre-excisable kits) improves the developmental potential of iPS cells and significantly increases their capacity to undergo directed differentiation *in vitro*. This is a step towards safer iPS cell technology for studying disease models and clinical therapies[2].

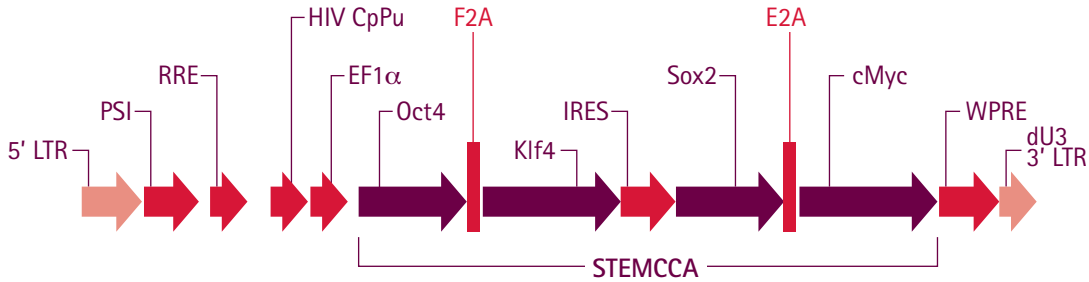
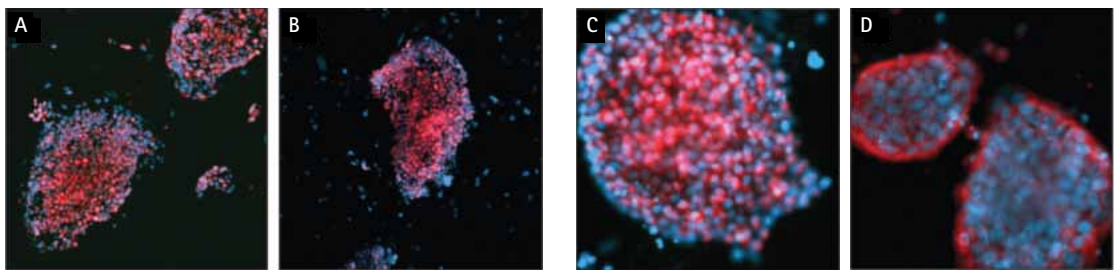


Figure 2. The STEMCCA vector is comprised of the transcription factors Oct-4, Klf4, SOX-2, and c-Myc (OKSM), separated by the self-cleaving 2A peptide and IRES sequences driven by the EF-1α constitutive promoter[1,2]. It is also available with flanking LoxP sites incorporated for Cre-mediated excision of the exogenous reprogramming transgenes.

Serum-containing culture

Serum-free culture



Neural differentiation medium

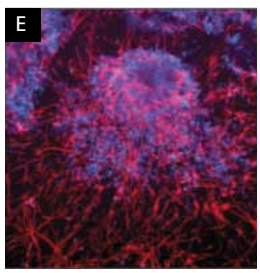


Figure 3. Successful reprogramming, serum-free culture, and neural differentiation of mouse embryonic fibroblasts after infection with single-vector Mouse STEMCCA Lentivirus (Cat. No. SCR530), as indicated by expression of characteristic pluripotency markers Oct-4 (A), Sox-2 (B), and SSEA-1 (not shown). Serum- and feeder-free culture (2 passages) of these iPS cells does not affect pluripotency, as can be seen by comparing Oct-4 expression in serum-containing (A) and serum-free (C) culture. These iPS cells cultured without serum also continued to express SSEA-1 (D) and Sox-2 (not shown). For neural differentiation, mouse iPS cells cultured in serum-free medium for 4 passages were differentiated for 11 days in ES2N Complete medium (Cat. No. SCM082). 80% of cells were βIII-tubulin positive (E).

STEMCCA Technology to Advance Your Research

STEMCCA kits are available in two formats, for reprogramming either rodent or human cells, and include lentivirus that expresses either mouse or human OKSM factors from a single polycistronic transcript. Both human and mouse STEMCCA lentivirus kits are available in constitutive and Cre/LoxP-regulated formats.

Whether mouse or human, the STEMCCA advantage means safer, more efficient and more consistently reproducable iPS cells than traditional systems.

Ordering Information

Description	Qty/pack	Catalogue No.
Human STEMCCA Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	30 µL lentivirus + Polybrene® transfection reagent	SCR544
Human STEMCCA Cre-Excisable Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	30 µL lentivirus + Polybrene transfection reagent	SCR545
Mouse STEMCCA Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	15 µL lentivirus + Polybrene transfection reagent	SCR510
Mouse STEMCCA Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	45 µL lentivirus + Polybrene transfection reagent	SCR530
Mouse STEMCCA Cre-Excisable Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	15 µL lentivirus + Polybrene transfection reagent	SCR511
Mouse STEMCCA Cre-Excisable Constitutive Polycistronic (OKSM) Lentivirus Reprogramming Kit	45 µL lentivirus + Polybrene transfection reagent	SCR531
Anti-SSEA-3	100 µg	MAB4303
Anti-TRA-1-60	100 µg	MAB4360

References

1 Sommer CA, *et al.* iPS cell generation using a single lentiviral stem cell cassette. *Stem Cells.* 2009 Mar;27(3): 543-9.

2 Sommer CA, *et al.* Excision of Reprogramming Transgenes Improves the Differentiation Potential of iPS Cells Generated with a Single Excisable Vector. *Stem Cells.* 2010 Jan;28(1): 64-74.

3 Takahashi K and Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell.* 2006 Aug 25;126(4): 633-676.

4 Okita, et al. Generation of germline-competent induced pluripotent stem cells. *Nature.* 2007 Jul 19;448(7151): 313-7.

5 Wernig M, *et al. In vitro* reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature.* 2007 Jul 19;448(7151): 318-24.

6 Takahashi K, *et al.* Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell.* 2007 Nov 30;131(5): 861-72.

7 Yu J, et al. Induced pluripotent stem cell lines derived from human somatic cells. *Science.* 2007 Dec 21;318(5858): 1917-20.

8 Stadtfeld M, *et al.* A reprogrammable mouse strain from gene-targeted embryonic stem cells. *Nature Methods.* 2010 Jan;7(1): 53-5.

9 Somers A, *et al.* Generation of transgene-free lung disease-specific human induced pluripotent stem cells using a single excisable lentiviral stem cell cassette. *Stem Cells.* 2010 Oct;28(10):1728-40.

Related Products

Human Reprogramming

Description	Qty/Pk	Catalogue No.
FibroGRO™ Xeno-Free Human Foreskin Fibroblasts	1 x 10^6 cells	SCC058
FibroGRO LS Complete Medium	500 mL	SCMF002
HEScGRO® Medium for Human ES Cell Culture	five 100 mL vials	SCM020
Recombinant Human basic FGF	50 ug	GF003
Alkaline Phosphatase Detection Kit	1 kit	SCR004
Anti-Human Oct-4 (clone 10H11.2)	100 µg	MAB4401
Anti-SSEA-4 (clone MC-813-70)	100 µg	MAB4304
Anti-TRA-1-81	100 µg	MAB4381

Mouse Reprogramming

Description	Qty/Pk	Catalogue No.
Primary Mouse Embryo Fibroblast Cells, not mitomycin-C-treated, strain CF1, passage 3	5 vials, 5-6 x 10^6 cells ea.	PMEF-CFL
ESGRO®-2i Medium	100 mL	SF016-100
	200 mL	SF016-200
ESGRO Complete™ Plus Clonal Grade Medium	100 mL	SF001-100P
	500 mL	SF001-500P
EmbryoMax Complete ES Cell Media w/ 15% FBS and mLIF	500 mL	ES-101-B
ESGRO mLIF Medium Supplement	10^6 units	ESG1106
	10^7 units	ESG1107
ES2N Medium for differentiating miPS cells into neurons	250 mL	SCM082
Alkaline Phosphatase Detection Kit	1 kit	SCR004
Anti-Mouse Oct-4 (clone 7F9.2)	100 µg	MAB4419
Anti-SOX-2	100 µg	AB5603
Anti-SSEA-1 (clone MC-480)	100 µg	MAB4301
Mouse STEMCCA Viral Gene Detection qPCR Multiplex Kit (Mouse)	100 reactions	SCR581
Amplifluor® Mouse Nanog JOE Primer Set	100 reactions	SCR588

To Place an Order or Receive Technical Assistance

In Europe, please call Customer Service:

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Germany: 01805 045 645 Italy: 848 845 645

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