Mouse iPS Cell Boost Supplement

Catalog No. SCM087

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures.

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Introduction

EMD Millipore offers a single lentiviral vector that enables the expression of a "stem cell cassette" (STEMCCA) comprised of the four Yamanaka transcription factors Oct-4, Klf4, Sox-2, and c-myc (OKSM) from a single polycistronic transcript [1]. STEMCCA reprogramming kits are available in mouse or human formats and include lentivirus that expresses either mouse or human OKSM factors, respectively. Both human and mouse STEMCCA kits are available in constitutive and Cre/LoxP-regulated formats [1-3].

Even with use of a single vector, however, reprogramming mouse somatic cells remains a highly inefficient and time-consuming process. Small molecules targeting specific signaling pathways have been shown to enhance the efficiency and/or replace transcription factors involved in somatic cell reprogramming. EMD Millipore has screened multiple chemical compounds and selected the compounds in this kit based on their effects on enhancement of reprogramming efficiency as assessed by the number and overall quality of mouse iPS colonies generated.

Product Description

EMD Millipore's Mouse iPS Cell Boost Supplement contains three proprietary small molecules in amounts sufficient to supplement over 250 mL mouse ES/iPS cell maintenance medium. When used in conjunction with the Mouse STEMCCA lentivirus reprogramming kits (SCR530, SCR531), the Mouse iPS Cell Boost Supplement enhanced the efficiency of mouse iPS colony formation by at least 3-fold. Mouse iPS Cell Boost Supplement can be used to enhance reprogramming efficiency in somatic cell lines that may be more refractory to reprogramming. Mouse iPS cells generated through use of the Mouse iPS Cell Boost Supplement display characteristic ES cell-like morphology, stained positive for alkaline phosphatase, expressed the correct mouse ES cell marker phenotype (SSEA-1 and Sox-2) and can be rapidly expanded in normal mouse ES cell culture conditions.

The Mouse iPS Cell Boost Supplement in combination with the Mouse STEMCCA lentivirus reprogramming kits has been validated on p3 mouse embryonic fibroblasts. Other cell types have not been tested and thus similar results can not be guaranteed.

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Kit Components

Contain sufficient amounts to supplement over 250 mL of normal mouse ES/iPS cell maintenance medium.

1. TGF-β RI Kinase Inhibitor II Supplement (1000X): (Part No. CS204458): One (1) vial containing 300 µL of the inhibitor in high quality DMSO. Store at -20°C. Aliquot into smaller working volumes. Avoid freeze thaw.
2. GSK3β Inhibitor Supplement (1000X): (Part No. CS204418): One (1) vial containing 300 µL of the inhibitor in high quality DMSO. Store at -20°C. Aliquot into smaller working volumes. Avoid freeze thaw.
3. Methylation Modulator-A Supplement (1000X): (Part No. CS204419): One (1) vial containing 300 µL of the inhibitor in water. Store at -20°C. Aliquot into smaller working volumes. Avoid freeze thaw.

Storage and Handling

Components of the Mouse iPS Cell Boost Supplement are stable for at least 6 months from date of receipt when stored at -20℃. Upon first thaw, cen trifuge the vial and gently mix the solution. Aliquot into smaller working volumes and freeze at -20℃ or -80℃. Upon addition of the components of the Mouse iPS Cell Boost Supplement to the media, filter the supplemented media with a 0.22 µM filtration unit. Supplemented media is good for up to two weeks when stored at 2-8℃.

Important Safety Note: Wear personal protective equipment when using this product. Avoid skin contact or ingestion of all chemicals used in this protocol. GSK3β Inhibitor Supplement (1000X) and TGF-β RI Kinase Inhibitor II Supplement (1000X) contain DMSO; avoid contact with eyes and skin.

Materials Required but Not Provided

1. Retro or lenti-virus based reprogramming systems. We recommend Mouse STEMCCA Lentivirus Reprogramming Kits (SCR530, SCR531)
2. 6-well plates, culture flasks, dishes (TC grade)
3. MEF expansion medium (see page 3)
4. PMEF cells, not mitomycin-C treated (Cat. No. PMEF-CFL)
5. Phosphate Buffered Saline (1X PBS) (Cat. No. BSS-1005-B)
6. EmbryoMax® 0.1% Gelatin Solution (Cat. No. ES-006-B)
7. EmbryoMax Complete ES Cell Media w/15% FBS and mLIF (Cat. No. ES-101-B)
8. Trypsin-EDTA Solution (0.25% Trypsin & 1 mM EDTA) (Cat. No. SM-2003-C)
9. Accutase™ Cell Dissociation Solution (Cat. No. SCR005)
10. FibroGRO™ LS Complete Medium (Cat. No. SCMF002)
11. PMEF cells, growth-arrested, mitomycin-C treated (Cat. No. PMEF-CF)

SECTION 1: REPROGRAMMING MOUSE EMBRYONIC FIBROBLASTS

Important note 1: The Mouse iPS Cell Boost Supplement is expected to work in other retro- and lentiviral based reprogramming systems, but has only been validated using the Mouse STEMCCA lentivirus kits (SCR530, SCR531, SCR518). Please follow the specific manufacturer's protocol for reprogramming.

***Important note 2*:** The following protocol has been optimized using early passage primary mouse embryo fibroblasts (MEFs) and should be used only as a **guide** to further optimize reprogramming of other somatic cells derived from rodents.

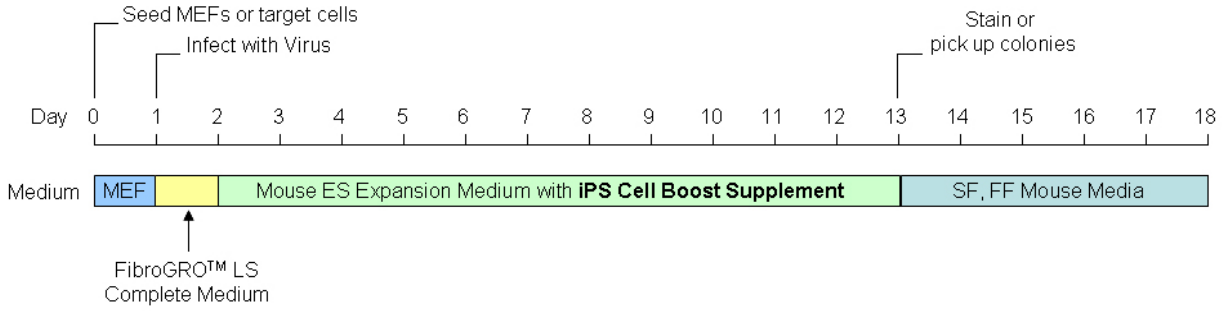

Figure 1. Time course schematic of reprogramming mouse somatic cells.

Day 0: Seeding proliferating MEFs or target rodent cells

1. Coat a sterile 6-well plate with 0.1% gelatin solution (Cat. No. ES-006-B). Use 2 mL volume per well. Incubate for at least 30 minutes before using. Aspirate the gelatin solution just before seeding the MEFs or target rodent cells.

2. Prepare 50 mL MEF Expansion Medium. Sterile filter with 0.22 µm filter.

Component	Quantity	Final Conc.	Millipore Cat. No.
DMEM High-Glucose Medium	44 mL		SLM-021-B
Fetal Bovine Serum	5.0 mL	10%	ES-009-B
L-Glutamine (200 mM)	0.5 mL	2 mM	TMS-002-C
Penicillin Streptomycin Solution (100X)	0.5 mL	1X	TMS-AB2-C

3. Seed 1 x 10^5 actively proliferating p3 mouse embryonic fibroblasts (Cat. No. PMEF-CFL) in 3 mL MEF Expansion media into each well of a 0.1% gelatin coated 6-well plate. Incubate overnight in a 37℃, 5% CO_2 incubator. It is recommended to use early passage MEFs.

Day 1: Virus Infection

4. Aspirate the MEF Expansion media and wash cells with 1X PBS buffer per well. Aspirate after the wash. Add 1 mL fresh FibroGRO™ LS Complete Medium (Cat. No. SCMF002) per well. Dilute 1 µL of Polybrene transfection reagent into 9 µL of sterile distilled water to create a 1:10 dilution. Add 5 µL of the diluted Polybrene transfection reagent to each well to be transduced. Final polybrene concentration should be 5 µg/mL. Set the plate aside in 37℃, 5% CO_2 incubator until ready to add the virus.

5. Using the equation provided below, determine the volume of virus required to achieve a multiplicity of infection (MOI) of at least 20. **Please make note of the titer as the viral titer may vary slightly from lot to lot.**

$$\text{Virus volume (µL) required} = \frac{\text{Number of MEFs seeded (from step 3)}}{\text{Virus Titer (IFU/mL)}} \times \frac{\text{Desired MOI}}{1\text{ mL}} \times 1000\ \mu L$$

Example: If the number of cells in the well at the time of transduction is 1×10^5, the viral titer is 3×10^8 IFU/mL, and a desired MOI is 20, then the volume of virus required is:

$$\frac{1 \times 10^5 \text{ cells}}{3 \times 10^8 \text{ IFU/mL}} \times \frac{20}{1\text{ mL}} \times 1000\ \mu L = *6.6\ \mu L \text{ virus required for 1 well of a 6-well plate}$$

***Note:** *Use the actual viral titer located on the label on the front of the manual in the equation above to determine the actual volume of virus to add.*

6. Thaw the reprogramming retro- or lentivirus at room temperature and quickly place the vial on ice after it is thawed. Quickly centrifuge the vial to spin down the contents. Keep the virus on ice and proceed immediately to the next step.

7. Add the required volume of thawed virus directly to the wells containing the attached MEFs (from Step 4). Gently rock the plate from side to side to thoroughly mix the virus onto the MEFs. Incubate overnight in a 37℃, 5% CO_2 incubator.

Day 2: Addition of Mouse iPS Cell Boost Supplement (Cat. No. SCM087)

8. Exchange the media with 3.0 mL Complete ES Cell Media with 15% FBS and LIF (Cat. No. ES-101-B). Add 3 µL **each** of TGF-β RI Kinase Inhibitor II Supplement (1000X) (Part No. CS204458), GSK3β Inhibitor Supplement (1000X) (Part No. CS204418) and Methylation Modulator-A Supplement (1000X) (Part No. CS204419).

   Each well should contain the following:

   3 mL Complete ES Cell Media with 15% FBS and LIF (Cat. No. ES-101-B)

   3 µL TGF-β RI Kinase Inhibitor II Supplement (1000X) (Part No. CS204458)

   3 µL GSK3β Inhibitor Supplement (1000X) (Part No. CS204418)

   3 µL Methylation Modulation-A Supplement (1000X) (Part No. CS204419)

   ~3.009 mL Total Volume

Day 4 – Day 13

9. Exchange the media with 3 mL fresh Complete ES Cell Media with 15% FBS and LIF (Cat. No. ES-101-B) containing 3 µL **each** of the components in Mouse iPS Cell Boost Supplement every other day for a total of 10-13 days. Mouse iPS cell colonies start to emerge around day 7-10.

10. Mouse iPS cell colonies can be selected and clonally expanded (typically around Day 12-14) when they reach an approximate size where the colony fits into the frame of a 10X Magnification view. ***Note: Once the mouse iPS cell colonies are established (typically day 12-14), it is no longer necessary to add the Mouse iPS Cell Boost Supplement to the media. Passage colonies as normal without the addition of the Mouse iPS Cell Boost Supplement.***

Representative Results

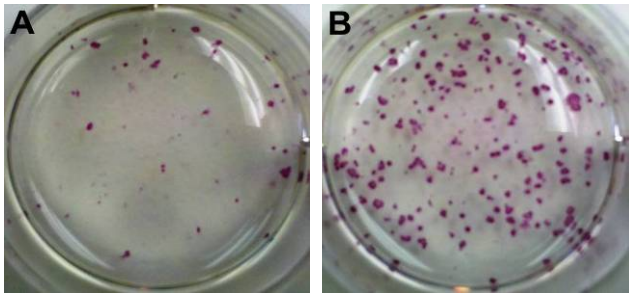

Figure 2. Mouse iPS colonies were generated from proliferating MEFs (passage 3) infected with the STEMCCA Cre-Excisable Constitutive Polycistronic (OKSM) Lentivirus. Lentiviral infection was performed with an MOI of 10 and 5 µg/mL Polybrene reagent. Mouse iPS Cell Boost Supplement (Cat. No. SCM087) was applied to the media one day after infection (**B**). Cells were fixed on Day 12 after infection and were stained using the Alkaline Phosphatase Detection Kit (Cat. No. SCR004). Considerably more iPS colonies emerged with the use of Mouse iPS Cell Boost Supplement (**B**) than in the plate containing the negative untreated control (**A**).

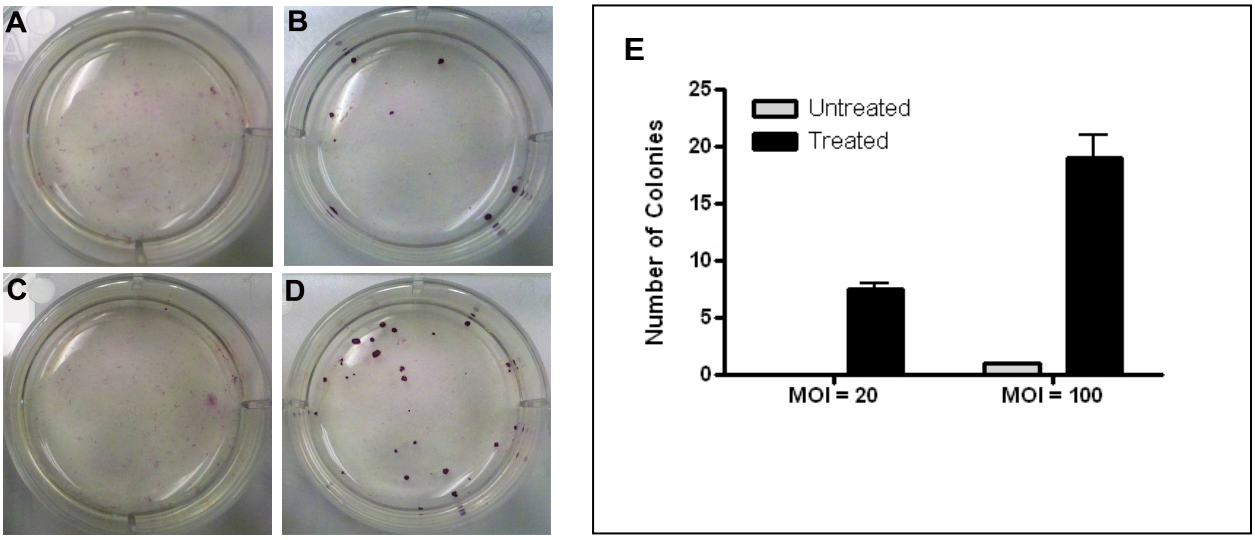

Figure 3. Mouse iPS Cell Boost Supplement can be used on somatic cell lines that are more difficult to reprogram. Mouse iPS colonies were generated from proliferating MEFs (passage 3) infected with the STEMCCA Cre-Excisable Constitutive Polycistronic (OKS) lentivirus which lacks the c-myc transcription factor (Cat. No. SCR518). Lentiviral infection was performed with an MOI of 20 (**A, B**) or 100 (**C, D**) and 5 µg/mL polybrene reagent. Mouse iPS Cell Boost Supplement was applied one day after infection (**B**, **D**) and maintained throughout the reprogramming process (i.e. day 12-14). Cells were fixed on day 12 after infection and were stained using the Alkaline Phosphatase Detection Kit (Cat. No. SCR004). Reprogramming was significantly impacted by the absence of the c-myc proto-oncogene. In untreated controls that contain only the OKS-loxP lentivirus and not the Mouse iPS Cell Boost Supplement **(A, C)**, colonies were not observed at MOI = 20 (**A**). Only by increasing virus MOI to 100, did 1 colony emerge (**C**). In contrast, the addition of Mouse iPS Cell Boost Supplement to the reprogramming process significantly increased the number of colonies that emerged from MOI of 20 (**B, E**) and 100 (**D, E**).

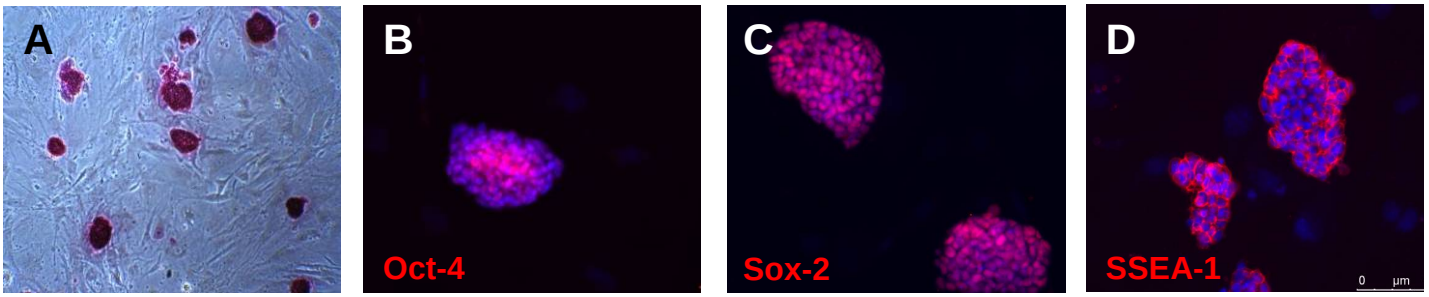

Figure 4. Mouse iPS cells derived from MEFs (passage 3) infected with the STEMCCA Cre-Excisable Constitutive Polycistronic (OKSM) Lentivirus and treated with Mouse iPS Cell Boost Supplement have cell morphology and staining characteristics of mouse ES cells. Lentiviral infection was performed with an MOI of 20 and 5 µg/mL Polybrene reagent. Mouse iPS Cell Boost Supplement was applied one day after infection and maintained throughout the culture until day 12-14. After 12-14 days, non-infected MEFs remained in a monolayer culture with no ES cell-like colonies observed while infected MEFs formed multilayered, tightly packed cells with well-defined borders in the presence of Mouse iPS Cell Boost Supplement. Four factor derived mouse iPS cells exhibit high alkaline phosphatase activity (**A**, Cat. No. SCR004) and expressed high levels of Oct-4 (**B**, Cat. No. MAB4419), Sox-2 (**C**, Cat. No. AB5603), and SSEA-1 (**D**, Cat. No. MAB4301). Cell nuclei were counterstained with DAPI (blue).

References

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