ReproRNA™-OKSGM

A Non-Integrating, Self-Replicating RNA Reprogramming Vector for Generating iPS Cells

Catalog #05930	1 Kit
Catalog #05931	12 µg
Catalog #05934	1 Kit

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Product Description

ReproRNA™-OKSGM is a non-integrating, self-replicating RNA-based reprogramming vector. This vector expresses Oct-3/4, Klf-4, Sox2, Glis1, c-Myc, and a puromycin-resistant cassette in a single construct.

- ReproRNA™-OKSGM is an RNA-based reprogramming system
- Non-integrating
- Non-viral
- Single transfection

The ReproRNA™-OKSGM Kit (Catalog #05930) includes ReproRNA™-OKSGM (Catalog #05931) and the Transfection Reagent Kit (Catalog #05934; ReproRNA™ Transfection Reagent and ReproRNA™ Transfection Supplement).

Product Information

PRODUCT NAME	CATALOG #	SIZE	STORAGE	SHELF LIFE
ReproRNA™-OKSGM	05931	12 µg	Store at -80°C.**	Stable for 9 months from date of manufacture (MFG) on label.
Transfection Reagent Kit	05934			
ReproRNA™ Transfection Reagent*†	05932	100 µL	Store at 2 - 8°C.	Stable for 6 months from date of receipt.
ReproRNA™ Transfection Supplement*†	05933	100 µL	Store at 2 - 8°C.	Stable for 6 months from date of receipt.

**Do not store at -20°C.
*This component is sold as part of the ReproRNA™-OKSGM Kit (Catalog #05930) and the Transfection Reagent Kit (Catalog #05934) and is not available for individual sale.
†Please refer to the Safety Data Sheet (SDS) for hazard information.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Corning® Matrigel® hESC-Qualified Matrix	Corning 354277
Tissue culture-treated 6-well plates	Corning 353046
Phosphate-Buffered Saline (PBS)	---
DMEM with 1000 mg/L D-glucose	36253
Fetal bovine serum (FBS)	---
MEM Non-Essential Amino Acid Solution (100X)	07600
L-Glutamine	07100
Advanced DMEM	Life Technologies 12491-015
B18R Recombinant Protein Carrier-Free	eBioscience 34-8185
Opti-MEM® I Reduced-Serum Medium	Life Technologies 31985-062
ReproTeSR™	05920
Puromycin (Dihydrochloride)	73342
Trypsin-EDTA (0.25%)	07901
Sterile microtubes	Sarstedt 72.730.005

Preparation of Reagents and Materials

Use sterile techniques to prepare the following reagents and materials.

1) FIBROBLAST CULTURE MEDIUM

The following example is for preparing 500 mL of Fibroblast Culture Medium. If preparing other volumes, adjust accordingly.

Combine the following:

- 440 mL DMEM with 1000 mg/L D-glucose
- 50 mL FBS
- 5 mL MEM Non-Essential Amino Acid Solution
- 5 mL 200 mM L-Glutamine

Pre-warm to room temperature (15 - 25°C) before use. Store Fibroblast Culture Medium at 2 - 8°C for up to 2 weeks.

2) GROWTH MEDIUM

The following example is for preparing 100 mL of Growth Medium. If preparing other volumes, adjust accordingly.

Combine the following:

- 89 mL Advanced DMEM
- 10 mL FBS
- 1 mL 200 mM L-Glutamine
- 40 µL 0.5 mg/mL B18R Recombinant Protein (final concentration 0.2 µg/µL)

Pre-warm to room temperature (15 - 25°C) before use. Store Growth Medium at 2 - 8°C for up to 1 week.

3) ReproRNA™ COCKTAIL

NOTE: Prepare ReproRNA™ Cocktail immediately before transfection.

12 µg of ReproRNA™ is provided (1 µg/µL), which is sufficient for reprogramming 12 wells of fibroblasts in a 6-well plate format. ReproRNA™-OKSGM is an RNA-based vector; use RNase- and DNase-free pipette tips and microtubes when preparing the ReproRNA™ Cocktail.

For each well (6-well plate format) of fibroblasts to be reprogrammed, combine the following components in the order shown in a sterile microtube:

- 1 µL ReproRNA™-OKSGM
- 100 µL Opti-MEM® I Reduced-Serum Medium
- 2 µL ReproRNA™ Transfection Supplement
- 2 µL ReproRNA™ Transfection Reagent

Pipette gently to mix after adding each component. Incubate ReproRNA™ Cocktail at room temperature (15 - 25°C) for exactly 5 minutes before immediately adding to cells. Volumes can be scaled up when reprogramming multiple wells of fibroblasts.

4) GROWTH MEDIUM + PUROMYCIN

NOTE: Prepare Growth Medium + Puromycin fresh daily, as required.

The following example is for preparing 12 mL of Growth Medium + Puromycin. If preparing other volumes, adjust accordingly.

Combine the following:

- 9.6 µL of a 1 µg/mL solution of puromycin (diydrochloride) (final concentration 0.8 ng/mL)
- 12 mL Growth Medium

5) ReproTeSR™ (**with** and **without** B18R)

NOTE: For preparation of complete ReproTeSR™ medium (**without** B18R), refer to the ReproTeSR™ Product Information Sheet (Document #DX20217), available on our website at www.stemcell.com or contact us to request a copy.

The following example is for preparing 100 mL of ReproTeSR™ **with** B18R. If preparing other volumes, adjust accordingly.

Combine the following:

- 40 µL 0.5 mg/mL B18R Recombinant Protein (final concentration 0.2 µg/µL)
- 100 mL complete ReproTeSR™ medium

Pre-warm to room temperature (15 - 25°C) before use. Store ReproTeSR™ **with** B18R at 2 - 8°C for up to 1 week.

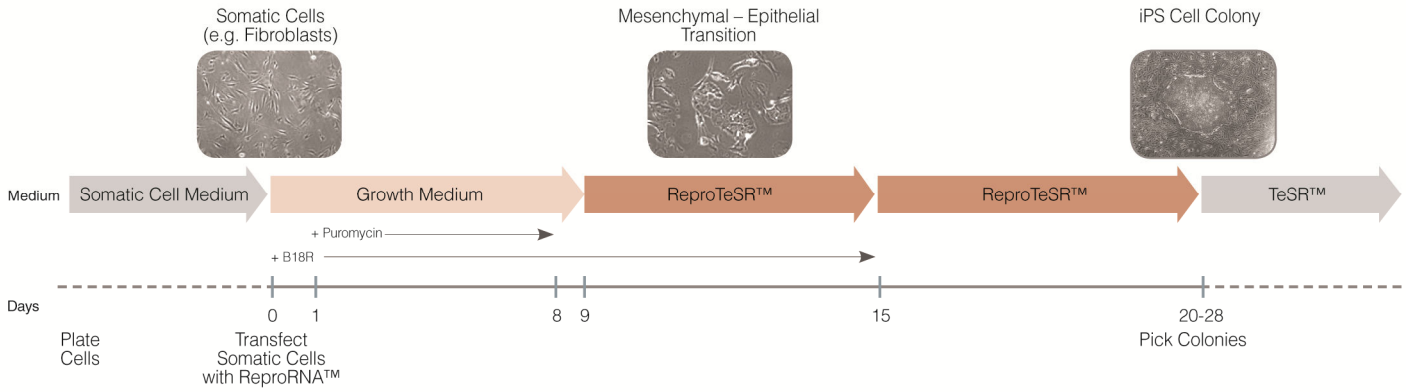

Figure 1. Timeline for Reprogramming with ReproRNA™-OKSGM

Somatic cells are transfected with ReproRNA™-OKSGM at day 0, and cultured in Growth Medium (containing puromycin). After 6 days of puromycin selection post-transfection, cells are cultured in ReproTeSR™ for the remainder of the reprogramming induction phase until iPS cell colonies emerge. B18R recombinant protein is also added during the first 2 weeks after transfection to inhibit the interferon response and increase cell viability. Typically, by day 20, iPS cell colonies are large enough to be isolated and propagated in *TeSR™ media.

*TeSR™ = TeSR™ family media (mTeSR™1, TeSR™2, TeSR™-E8™)

Directions for Use

Please read the entire protocol before proceeding. Use sterile techniques when performing the following protocols.

A. CULTURE OF HUMAN DERMAL FIBROBLASTS

Use low-passage (passage 2 - 5) human dermal fibroblasts for reprogramming experiments. Extended passaging of fibroblasts will decrease reprogramming efficiency.

The following instructions are for 1 well of a 6-well plate. If using other cultureware, adjust volumes accordingly.

1. Plate low-passage fibroblasts (3 - 5000 cells/cm^2) in Fibroblast Culture Medium and incubate at 37°C and 5% CO_2.
2. Every 2 days, aspirate medium and replace with 2 mL fresh Fibroblast Culture Medium.
3. When the culture is approximately 85% confluent, passage with Trypsin-EDTA (0.25%) as follows:
   a. Aspirate Fibroblast Culture Medium. Rinse twice with phosphate-buffered saline (PBS).
   b. Add 1 mL Trypsin-EDTA (0.25%).
   c. Incubate at 37°C and 5% CO_2 for 2 - 5 minutes or until fibroblasts have detached.
   d. Add 1 mL Fibroblast Culture Medium to inactivate trypsin. Transfer cell suspension to a conical tube.
   e. Centrifuge at 300 x *g* for 5 minutes. Remove and discard supernatant and resuspend cells in fresh Fibroblast Culture Medium.
   f. Plate fibroblasts at a split ratio of 1:4 to 1:6. Incubate at 37°C and 5% CO_2 until ready to reprogram the cells.

B. RNA TRANSFECTION PROTOCOL

The protocol below describes the reprogramming of human dermal fibroblasts in 1 well of a 6-well plate. If using other cultureware, adjust volumes accordingly.

Harvesting and Plating Fibroblasts

Coat cultureware with Corning® Matrigel® hESC-Qualified Matrix and bring to room temperature (15 - 25°C) for at least 30 minutes prior to use.

NOTE: For complete instructions on coating plates with Corning® Matrigel®, please refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™1 (Document #29106) or TeSR™-E8™ (Document #29267), available on our website at www.stemcell.com or contact us to request a copy.

1. Harvest fibroblasts (from section A) with Trypsin-EDTA (0.25%) as described in section A, steps 3a - 3d.
2. Centrifuge at 300 x *g* for 5 minutes. Remove and discard supernatant and resuspend cells in fresh Fibroblast Culture Medium at 5 x 10^4 cells/mL.
3. Add 2 mL of the cell suspension (1 x 10^5 cells) to 1 well of a Corning® Matrigel®-coated 6-well plate.
4. Incubate at 37°C and 5% CO_2 overnight to allow fibroblasts to attach to Corning® Matrigel®.

Transfection

DAY 0

5. Aspirate Fibroblast Culture Medium from each well and wash with 2 mL sterile PBS per well.
6. Add 1 mL Growth Medium to each well. Incubate at 37°C and 5% CO_2 while preparing ReproRNA™ Cocktail (step 5).
7. Prepare ReproRNA™ Cocktail in a sterile microtube for each well of fibroblasts to be reprogrammed. Incubate at room temperature (15 - 25°C) for 5 minutes.
8. Immediately add ReproRNA™ Cocktail dropwise to each well containing fibroblasts. Gently rock the plate back and forth and side-to-side to ensure the ReproRNA™ Cocktail is evenly distributed throughout the entire well.
9. Incubate at 37°C and 5% CO_2 overnight.

DAY 1

10. Aspirate Growth Medium containing ReproRNA™ Cocktail and add 2 mL Growth Medium + Puromycin per well.

    NOTE: The addition of Growth Medium + Puromycin removes fibroblasts that have not been transfected with ReproRNA™-OKSGM.
11. Incubate at 37°C and 5% CO_2 overnight.

DAY 3 - 7

12. Perform a daily medium change with 2 mL of fresh Growth Medium + Puromycin per well. Monitor fibroblasts for cell morphology and survival. Incubate at 37°C and 5% CO_2.

DAY 8

13. Aspirate medium and add 2 mL Growth Medium (without puromycin) per well. Incubate at 37°C and 5% CO_2 overnight.

DAY 9

14. Aspirate Growth Medium and add 2 mL ReproTeSR™ **with** B18R per well. Incubate at 37°C and 5% CO_2 overnight.

DAY 10 - 14

15. Perform daily medium changes with ReproTeSR™ **with** B18R. Incubate at 37°C and 5% CO_2.

DAY 15 - 28

16. Perform daily medium changes with ReproTeSR™ (**without** B18R) until iPS colonies form and are ready to be manually isolated. iPS colonies typically arise between 15 - 28 days post-transfection of ReproRNA™-OKSGM.
17. Manually isolate putative iPS cell colonies. Use either a 22 - 25 gauge needle or a pulled glass pipette to cut the putative iPS cell colony into small fragments. Then use a 200 µL micropipette with a filtered pipette tip to scrape and aspirate colony fragments.

    NOTE: If there are many untransfected, partially reprogrammed and/or differentiated cells surrounding the putative iPS cell colony, these may need to be scraped away prior to isolating the iPS cell colony.
18. Immediately plate iPS cell colony fragments on cultureware coated with desired matrix (e.g. Corning® Matrigel®) and containing iPS cell maintenance medium (e.g. mTeSR™1 [Catalog #05850] or TeSR™-E8™ [Catalog #05940]).

    NOTE: To facilitate the initial attachment of iPS cell colony fragments, add Y-27632 (Dihydrochloride; Catalog #72302) to the maintenance medium at a final concentration of 10 µM. After 24 hours, replace the maintenance medium (without Y-27632).
19. Incubate at 37°C and perform iPS cell maintenance medium changes as appropriate.

    NOTE: For complete instructions on how to maintain iPS cells using mTeSR™1 or TeSR™-E8™, refer to the Technical Manual: Maintenance of Human Pluripotent Stem Cells in mTeSR™1 (Document #29106) or TeSR™-E8™ (Document #29267), available on our website at www.stemcell.com or contact us to request a copy.

References

Yoshioka N et al. (2013) Efficient generation of human iPSCs by a synthetic self-replicative RNA. Cell Stem Cell 13(2): 246–54.