

# ReproRNA™-OKSGM

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



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

## 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® 1 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 (dihydrochloride) (final concentration 0.8 ng/mL)
- 12 mL Growth Medium

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

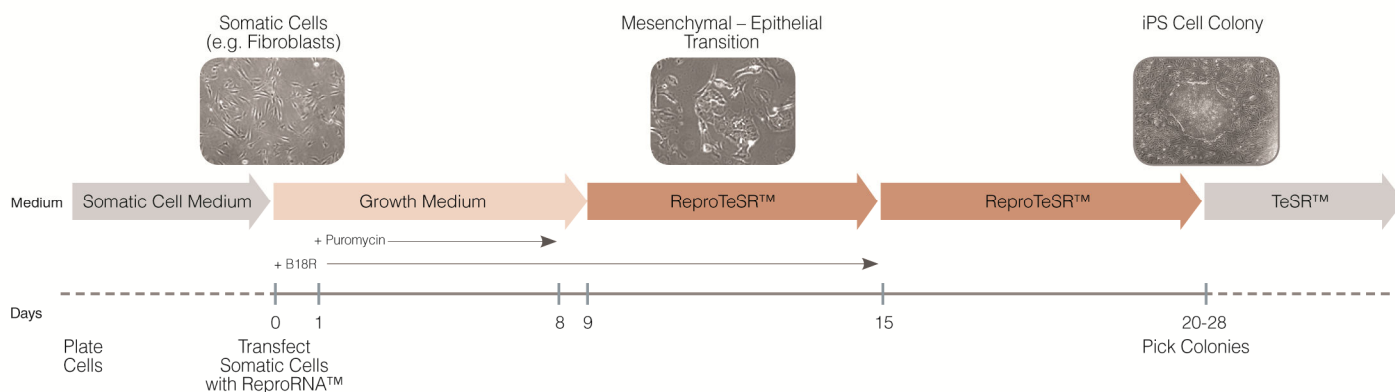
NOTE: For preparation of complete ReproTeSR™ medium (**without** B18R), refer to the ReproTeSR™ Product Information Sheet (Document #DX20217), available on our website at [www.stemcell.com](http://www.stemcell.com) or contact us to request a copy.

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<sup>2</sup>) in Fibroblast Culture Medium and incubate at 37°C and 5% CO<sub>2</sub>.
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<sub>2</sub> 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<sub>2</sub> 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](http://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<sup>4</sup> cells/mL.
3. Add 2 mL of the cell suspension (1 x 10<sup>5</sup> cells) to 1 well of a Corning® Matrigel®-coated 6-well plate.
4. Incubate at 37°C and 5% CO<sub>2</sub> overnight to allow fibroblasts to attach to Corning® Matrigel®.

## Transfection

### DAY 0

- Aspirate Fibroblast Culture Medium from each well and wash with 2 mL sterile PBS per well.
- Add 1 mL Growth Medium to each well. Incubate at 37°C and 5% CO<sub>2</sub> while preparing ReproRNA™ Cocktail (step 5).
- 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.
- 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.
- Incubate at 37°C and 5% CO<sub>2</sub> overnight.

### DAY 1

- 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.
- Incubate at 37°C and 5% CO<sub>2</sub> overnight.

### DAY 3 - 7

- 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<sub>2</sub>.

### DAY 8

- Aspirate medium and add 2 mL Growth Medium (without puromycin) per well. Incubate at 37°C and 5% CO<sub>2</sub> overnight.

### DAY 9

- Aspirate Growth Medium and add 2 mL ReproTeSR™ **with** B18R per well. Incubate at 37°C and 5% CO<sub>2</sub> overnight.

### DAY 10 - 14

- Perform daily medium changes with ReproTeSR™ **with** B18R. Incubate at 37°C and 5% CO<sub>2</sub>.

### DAY 15 - 28

- 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.
- 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.
- 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).
- 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](http://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.

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