Versatility and performance of Lipofectamine Stem Transfection Reagent

Introduction

The ability of stem cells to self-renew and differentiate into various specialized cell types promises to contribute greatly to future applications in regenerative medicine and to the development of novel therapeutic treatments. Technologies enabling the genetic manipulation of stem cells support preclinical and clinical research to develop new therapies using gene correction and tissue replacement techniques. The ability to subsequently isolate and expand stem cell lines under feeder-free culture conditions using Gibco™ StemFlex™ and Essential 8™ media, for example, further enables the advancement of stem cell research from the bench to the clinic. However, resistance of stem cells to transfection, due to the sensitivity of the cells, has been a major challenge to overcome. Transfecting stem cells without inhibiting cell viability and cell growth has been shown to be difficult; the main challenge lies in the delivery method, which must have little to no effect on the viability and function of the cell in order to ensure reliable downstream assay results. Research applications such as gene editing, gene expression, and directed differentiation all require the ability to deliver a variety of constructs into stem cells. Advances in gene editing using the CRISPR-Cas9 system also require the ability to deliver large plasmid constructs or a combination of DNA, RNA, and Cas9 ribonucleoprotein (RNP) complexes.

The goal of this study was to illustrate the versatility of Invitrogen™ Lipofectamine™ Stem Transfection Reagent, which was developed specifically for stem cells, to co-deliver multiple payloads in addition to large plasmid constructs with high transfection efficiency. Lipofectamine Stem reagent can be used to transfect a wide range of stem cell types with superior efficiency while supporting continued proliferation without inducing differentiation. Figures 1 and 2 provide an overview of additional options in our transfection portfolio for various cell types and payloads.

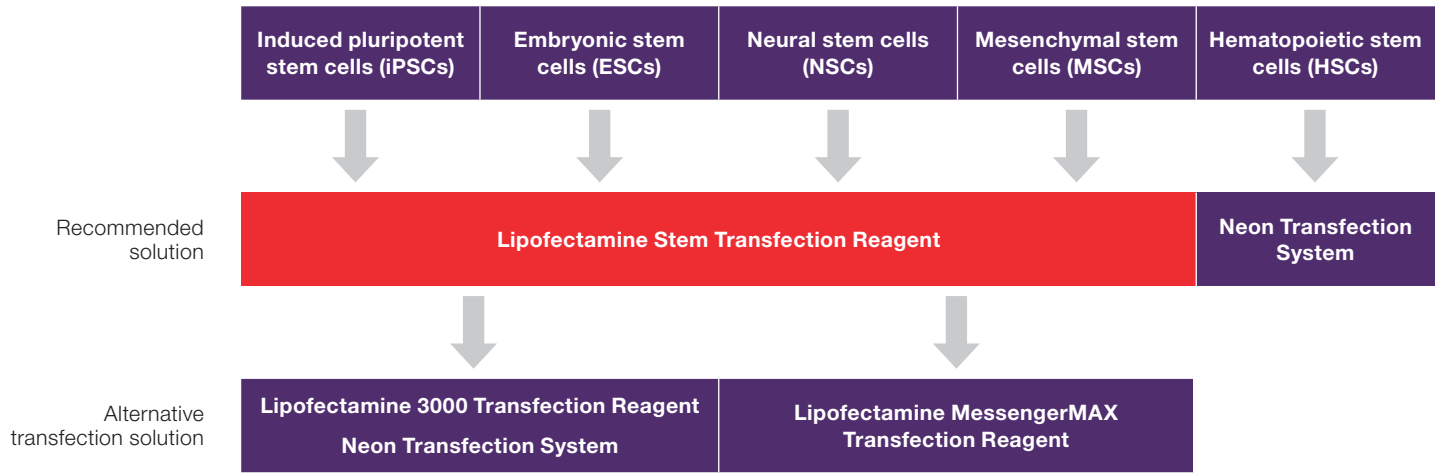


Figure 1. Our recommended gene delivery solutions for human iPSCs, ESCs, NSCs, and MSCs. Additional Invitrogen™ Lipofectamine™ reagents, as well as the Invitrogen™ Neon™ Transfection System for electroporation, are available as alternative solutions.

Transfection method	Recommended payloads				Transfection efficiency by cell type			
	DNA	mRNA	RNP (Cas9 protein)	Co-delivery	iPSC	ESC	NSC	MSC
Lipofectamine Stem reagent	✓	✓	✓	✓	■■■■	■■■■	■■■■	■■□□
Lipofectamine 3000 reagent	✓	✓		✓	■■■□	■■■□	■■□□	NA
Lipofectamine MessengerMAX reagent		✓			■■■□	■■■□	■■□□	■■□□
Neon Transfection System	✓	✓	✓	✓	■■■■	■■■■	■■■□	■■■■
Lipofectamine CRISPRMAX reagent			✓		■■■□	Not tested	Not tested	Not tested

Figure 2. Transfection selection guide for stem cells. Recommended payloads by transfection method, and transfection efficiency by cell type, are shown. Higher numbers of blocks represent higher efficiency.

Results

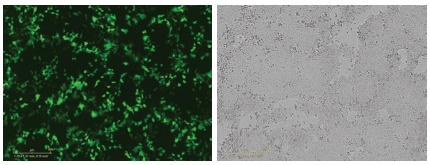
High-efficiency DNA transfection in human stem cells—PSCs, NSCs, and MSCs

Lipofectamine Stem reagent efficiently delivers small and large DNA plasmids into human PSCs (ESCs and iPSCs), NSCs, and MSCs with high efficiency (Figure 3). Transfection with large plasmids has traditionally been challenging, especially with a transfection reagent. Applications like gene editing frequently require large plasmids encoding gene-editing constructs or template DNA, and often require electroporation to transfect cells. Lipofectamine Stem reagent provides a complementary alternative to electroporation for introducing a wide range of plasmid DNA into stem cells, in addition to being gentler on cells. Lipofectamine Stem reagent is also compatible with a variety of cell culture media, including feeder-free cell culture systems (Figure 4).

A

iPSCs

Experimental condition	Recommendation
Delivery platform	Lipofectamine Stem reagent, 1 µL/well
Plate format	24-well plate
DNA	GFP plasmid, 500 ng/well
Medium	Essential 8 Medium
Extracellular matrix	Vitronectin
Cell density	50,000 cells/well



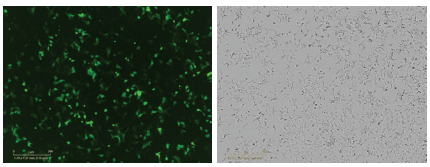
iPSCs, GFP plasmid

Transfection efficiency: 75%

B

iPSC-derived NSCs

Experimental condition	Recommendation
Delivery platform	Lipofectamine Stem reagent, 1 µL/well
Plate format	24-well plate
DNA	GFP plasmid, 500 ng/well
Medium	StemPro NSC SFM
Extracellular matrix	Geltrex matrix
Cell density	75,000 cells/well



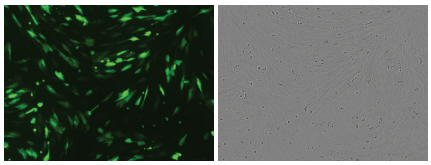
NSCs, GFP plasmid

Transfection efficiency: 60%

C

MSCs

Experimental condition	Recommendation
Delivery platform	Lipofectamine Stem reagent, 1 µL/well
Plate format	24-well plate
DNA	GFP plasmid, 500 ng/well
Medium	MesenPRO RS Medium
Extracellular matrix	CTS CELLstart Substrate
Cell density	25,000 cells/well

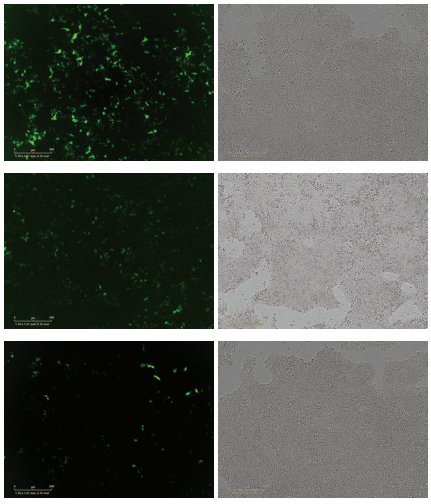


Adipose-derived MSCs, GFP plasmid

Transfection efficiency: 47%

D

ESCs: transfection with large DNA constructs



H9 ESCs in StemFlex Medium

Lipofectamine Stem reagent

11 kb EF1α-GFP DNA plasmid

Transfection efficiency: 76%

H9 ESCs in mTeSR1 Medium

Lipofectamine Stem reagent

11 kb EF1α-GFP DNA plasmid

Transfection effiiciency: 44%

H9 ESCs in mTeSR1 Medium

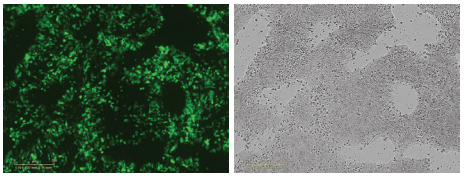
Other supplier's reagent

11 kb EF1α-GFP DNA plasmid

Transfection efficiency: 5%

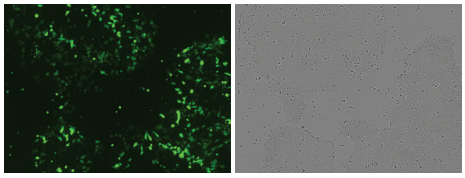
Figure 3. Transfection of human stem cells with small and large DNA plasmids, using Lipofectamine Stem Transfection Reagent. High-efficiency transfection with small DNA plasmids is shown in **(A)** iPSCs, **(B)** NSCs, and **(C)** MSCs. **(D)** H9 ESCs were transfected with an 11 kb DNA plasmid using Lipofectamine Stem reagent (top, middle), with significantly higher efficiency than with another leading supplier's reagent (bottom).

A



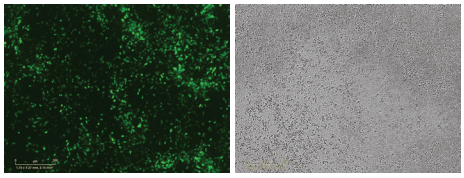
Transfection efficiency: 75%

B



Transfection efficiency: 65%

C

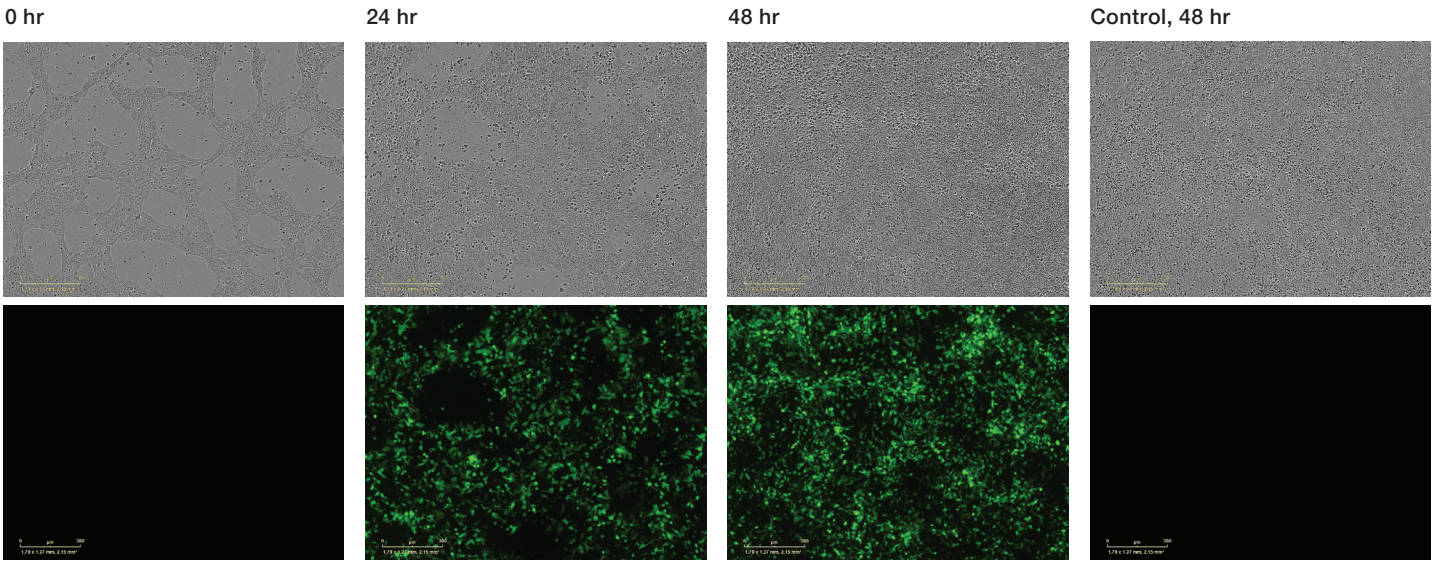


Transfection efficiency: 59%

Figure 4. Lipofectamine Stem reagent is compatible with a variety of media. Results show human PSCs at 38–44 hours posttransfection. Human iPSCs plated in 24-well plates were transfected with 1 or 2 µL Lipofectamine Stem reagent in **(A)** Essential 8 Medium on vitronectin, **(B)** StemFlex Medium on Gibco™ Geltrex™ matrix, and **(C)** mTeSR™1 Medium (STEMCELL Technologies) on Geltrex matrix.

Gentle on cells—keeps stem cells viable and healthy
Transfecting stem cells without inhibiting growth and viability can be challenging due to the sensitivity of these cells. The transfection process requires a balance between introducing foreign nucleic acids into a cell and not killing the cell in the process. Lipofectamine Stem reagent delivers low amounts of nucleic acid with high efficiency, allowing stem cells to stay healthy and continue proliferating without inducing differentiation. Pluripotent stem cells continue to proliferate with continuous transfection, reaching near-confluency by 48 hours (Figure 5).

A



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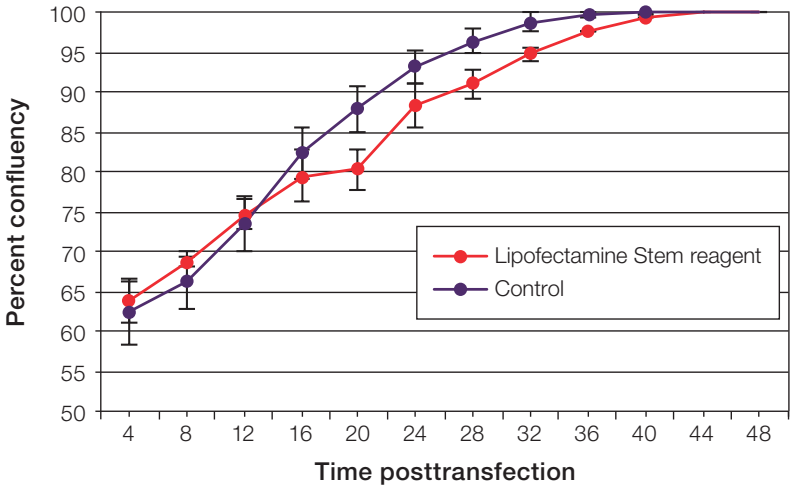


Figure 5. Lipofectamine Stem reagent maintains healthy, proliferating stem cells during transfection. (A) Human iPSCs cultured in feeder-free Essential 8 Medium were either transfected with 1 µL Lipofectamine Stem reagent or not transfected (control). Cells remained healthy and viable with normal morphology and continued to proliferate with continuous transfection, reaching confluency by 48 hr. **(B)** Confluency of transfected and untransfected control cells.

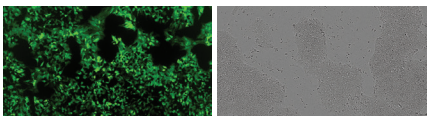
Advantages of mRNA transfection with Lipofectamine Stem reagent

Transient delivery of exogenous RNA into cells has been shown to be a safer alternative to the methods that rely on exogenous DNA or viral vectors. In contrast to DNA transfection, RNA transfection does not pose a risk of integration into the host genome, which can cause insertional mutagenesis and activation of oncogenes. Transfection with messenger RNA (mRNA) is a useful platform for manipulating cell genotype and phenotypes by gene editing and transcription factor–directed differentiation. Having control over the timing, dosage, and stoichiometry of transgene delivery provides a precise way to drive foreign protein production in stem cells.

A

ESCs

Experimental condition	Recommendation
Delivery platform	Lipofectamine Stem reagent, 1 µL/well
Plate format	24-well plate
mRNA	GFP mRNA, 250 ng/well
Medium	Essential 8 Medium
Extracellular matrix	Vitronectin
Cell density	50,000 cells/well

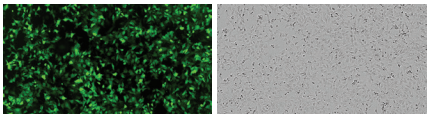


H9 ESCs, GFP mRNA
Transfection efficiency: 79%

B

iPSC-derived NSCs

Experimental condition	Recommendation
Delivery platform	Lipofectamine Stem reagent, 1 µL/well
Plate format	24-well plate
mRNA	GFP mRNA, 250 ng/well
Medium	StemPro NSC SFM
Extracellular matrix	Geltrex matrix
Cell density	75,000 cells/well

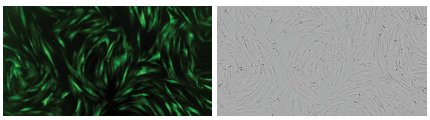


NSCs, GFP mRNA
Transfection efficiency: 80%

C

MSCs

Experimental condition	Recommendation
Delivery platform	Lipofectamine Stem reagent, 1 µL/well
Plate format	24-well plate
mRNA	GFP mRNA, 250 ng/well
Medium	CTS StemPro MSC SFM
Extracellular matrix	CTS CELLstart Substrate
Cell density	25,000 cells/well



MSCs, GFP mRNA
Transfection efficiency: 40%

Figure 6. High-efficiency transfection of human stem cells with mRNA using Lipofectamine Stem reagent. (A) H9 ESCs and **(B)** iPSC-derived NSCs were transfected with GFP mRNA (modified). **(C)** MSCs were transfected with GFP mRNA (modified). The cells were examined for GFP expression 24 and 48 hours posttransfection.

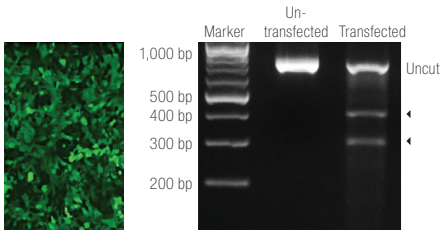
Efficient co-delivery of Cas9 RNP complexes and DNA

Research applications such as gene editing require the ability to deliver a variety of payloads into cells. Lipofectamine Stem reagent can be used to efficiently co-deliver RNP complexes of Cas9 protein with guide RNA (gRNA) into iPSCs, NSCs, and MSCs (Figure 7) to support high-efficiency, targeted insertions or deletions (indels). Single-stranded DNA constructs can also be mixed with Cas9 RNP complexes to promote homology-directed repair (HDR) and introduce targeted genomic sequences.

A

iPSCs

Experimental condition	Recommendation
Delivery platform	Lipofectamine Stem reagent, 1 µL/well
Plate format	24-well plate
Cas9 RNP	500 ng Cas9 protein/125 gRNA + 50 ng GFP mRNA
Medium	Essential 8 Medium
Extracellular matrix	Vitronectin
Cell density	50,000 cells/well

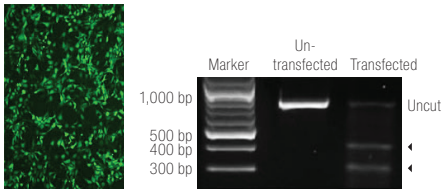


iPSCs
Cas9 RNP + GFP mRNA
Transfection efficiency: 90%

B

iPSC-derived NSCs

Experimental condition	Recommendation
Delivery platform	Lipofectamine Stem reagent, 1 µL/well
Plate format	24-well plate
Cas9 RNP	500 ng Cas9 protein/125 gRNA + 50 ng GFP mRNA
Medium	StemPro NSC SFM
Extracellular matrix	Geltrex matrix
Cell density	75,000 cells/well



NSCs
Cas9 RNP + GFP mRNA
Transfection efficiency: 87%

Figure 7. Transfection with Lipofectamine Stem reagent supports high-efficiency gene editing in human stem cells. (A) iPSCs were cotransfected with Cas9 RNP targeting the *EMX1* gene, along with GFP mRNA; **(B)** iPSC-derived NSCs in suspension were cotransfected with Cas9 RNP targeting the *EMX1* gene, along with GFP mRNA. Genomic PCR products of transfected PSCs (gel in **A**) and NSCs (gel in **B**) were analyzed by a T7 endonuclease I assay (T7 endo) to detect cleavage of the *EMX1* gene. Arrowheads denote cleavage bands.

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Conclusions

The optimal tool for mediating gene delivery into cells often depends on the payload that is to be delivered (DNA, RNA, or RNP), the size of the vector construct, and the cell type being transfected. Here we demonstrate the utility of Lipofectamine Stem Transfection Reagent as our most versatile and preferred choice for delivering DNA, mRNA, and Cas9 RNP into human PSCs (iPSCs and ESCs), NSCs, and MSCs. Lipofectamine Stem reagent was designed specifically for stem cells and confers low cytotoxicity, enabling researchers to achieve optimal transfection efficiencies without inhibiting cell viability or altering cell growth—supporting continued proliferation without inducing differentiation. Moreover, this reagent is compatible with various types of media, including feeder-free culture systems, adding versatility and flexibility to current stem cell culture workflows.

Find out more at **thermofisher.com/lipofectaminestem**

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