

	Contents	Catalog Number	Size
		▪ STEM00001	0.1 mL
		▪ STEM00003	0.3 mL
		▪ STEM00008	0.75 mL
		▪ STEM00015	1.5 mL

	Storage	Store at 4°C (do not freeze).
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	Required materials	<ul style="list-style-type: none"> ▪ Opti-MEM™ I Reduced Serum Medium ▪ Microcentrifuge tubes
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	Timing	<p>Preparation: 10 minutes</p> <p>Incubation: 10 minutes</p> <p>Final incubation: 1–2 days</p>
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	Product description	<ul style="list-style-type: none"> ▪ Lipofectamine™ Stem Reagent is a proprietary formulation for transfecting nucleic acids into a wide range of human stem cells.
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		<ul style="list-style-type: none"> ▪ Lipofectamine™ Stem Reagent should be made in Opti-MEM™ I Reduced Serum Medium and can be added directly to cells in culture growing with or without serum. ▪ It is not necessary to remove transfection complexes. Additional medium may be added the following day to stem cell cultures without interfering with the transfection.
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	Important guidelines	<ul style="list-style-type: none"> ▪ The amount of Lipofectamine™ Stem Reagent required for optimal transfection depends upon the amounts of stem cells plated and DNA used (See recommended amounts). <ul style="list-style-type: none"> ▫ Proliferating stem cell culture need room to expand, so plate the recommended starting cell numbers to achieve 30–60% confluency on the day of transfection. ▫ Using lower amounts of DNA, mRNA, or ribonucleoprotein (RNP) complexes can yield improved expression, while minimizing risks of cytotoxicity from excess foreign constructs.
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	Online resources	Visit our product page for additional information and cell specific protocols. For support, visit thermofisher.com/support .
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Protocol outline

- Plate cells so they will be 30–60% confluent at the time of transfection.
- Prepare transfection complexes.
- Add transfection complexes to cells.

Cell seeding recommendations for various stem cell models

Stem Cell Type	96-well	24-well	6-well
Human Pluripotent	1–1.5 × 10 ⁴ cells	5–7.5 × 10 ⁴ cells	2.5–3.75 × 10 ⁵ cells
Human Neural	1.5 × 10 ⁴ cells	7.5 × 10 ⁴ cells	3.75 × 10 ⁵ cells
Human Mesenchymal	0.5 × 10 ⁴ cells	2.5 × 10 ⁴ cells	1.25 × 10 ⁵ cells

DNA transfection

See page 2 to view a typical DNA transfection procedure.

Component	96-well	24-well	6-well
Final DNA per well	50–100 ng	250–500 ng	1.25–2.5 µg
Final Lipofectamine™ Stem Reagent per well	0.2–0.4 µL	1.0–2.0 µL	5.0–10 µL

mRNA Transfection

mRNA can be transfected in a 24-well plate with 1 or 2 µL Lipofectamine™ Stem Reagent complexed with 250 ng to 500 ng of mRNA per well.

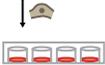
Ribonucleoprotein (RNP) Transfection

RNP complexes can be transfected into stem cells in a 24-well plate by combining 0.5 to 1.5 µg of GeneArt™ Platinum™ Cas9 nuclease with 125 to 375 ng of gRNA and then adding 1 to 2 µL of Lipofectamine Stem™ Transfection Reagent per reaction for each well.

Limited Product Warranty and Disclaimer Details

Lipofectamine™ Stem Transfection Reagent Protocol:

To optimize transfection of your stem cells in your culture conditions, we recommend starting with two amounts of DNA and two amounts of Lipofectamine™ Stem Transfection Reagent following the chart below. Volumes are listed on a per well basis in a 24-well format. Adjust the final amounts of components according to your experimental design and cell culture format. For additional information on setting up mRNA or RNP transfections see thermofisher.com/lipofectaminestem.

Timeline		Step	Procedure details				
Day 0	1 	Seed cells to be 30–60% confluent at transfection	Adherent stem cells: 2.5–7.5 x 10 ⁴ per well in 4 wells of a 24-well plate				
Day 1	2 	Dilute 2 amounts of Lipofectamine™ Stem Reagent in Opti-MEM™ I Medium	Component	Well number			
				1	2	3	4
	3 	Dilute 2 amounts of DNA in Opti-MEM™ I Medium	Opti-MEM™ I Medium	25 µL	25 µL	25 µL	25 µL
			DNA	250 ng	500 ng	250 ng	500 ng
	4 	Add diluted DNA to diluted Lipofectamine™ Stem Reagent (1:1 ratio)	Diluted DNA volume	25 µL	25 µL	25 µL	25 µL
			Diluted Lipofectamine™ Stem Reagent volume	25 µL	25 µL	25 µL	25 µL
5 	Incubate	Incubate for 10 minutes at room temperature.					
6 	Add DNA-lipid complex to cells	Component	Well number				
			1	2	3	4	
		DNA-lipid complex per well	50 µL	50 µL	50 µL	50 µL	
		Final DNA used per well	250 ng	500 ng	250 ng	500 ng	
7 	Visualize/analyze transfected cells	Final Lipofectamine™ Stem Reagent used per well	1 µL	1 µL	2 µL	2 µL	
		Incubate and monitor transfected stem cells at 37°C for 1–2 days.					