Transfection of neural stem cells with Lipofectamine Stem Transfection Reagent in StemPro medium

NSC media, passaging reagents, and complexation medium

Component	Cat. No.
StemPro NSC SFM	A1050901
CTS GlutaMAX-I Supplement	A1286001
Geltrex LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix	A1413302
Dulbecco's Phosphate Buffered Saline (DPBS) without calcium and magnesium	14190144
StemPro Accutase Cell Dissociation Reagent	A1110501
Opti-MEM I Reduced Serum Medium	31985062

Starting with undifferentiated human primary neural stem cells (NSCs) or pluripotent stem cell (PSC)–derived NSCs, expanded in a defined culture system such as Gibco™ StemPro™ NSC Serum-Free Medium (SFM) on Gibco™ Geltrex™ matrix, is ideal for efficient transfection.

Passaging

- Maintain NSCs in the format of your choice, such as 6-well plates, 60 cm dishes, T-25 flasks, or T-75 flasks coated with Geltrex matrix, in StemPro SFM. Propagating NSCs in T-25 flasks and transfecting in 24-well plates are convenient formats used in this protocol.
- Passage NSCs every 3 to 5 days at 90–100% confluence.
- Use Gibco™ StemPro™ Accutase™ Cell Dissociation Reagent to generate a single-cell suspension of NSCs for both expansion and seeding for transfection.

Precoating 24-well plates with Geltrex matrix for transfection

1. Prepare a 1:100 dilution of Geltrex matrix in cold Gibco™ DMEM/F-12 with Gibco™ GlutaMAX™ Supplement (Cat. No. 10565).
2. Add 300 µL of diluted Geltrex matrix to each well of a 24-well plate and incubate at 37°C for ≥1 hour, before use.

- **Tip:** Geltrex matrix–coated plates can be prepared ahead of time and stored for up to 2 weeks at 4°C. Equilibrate at room temperature for 1 hour before plating cells.

Seeding cells for transfections

1. When NSC cultures are ~90–100% confluent, remove the StemPro NSC SFM.

2. Wash NSCs once with 10 mL of DPBS without calcium and magnesium; aspirate the medium and discard.

3. Add 1 mL of room-temperature StemPro Accutase reagent to each T-25 flask, swirl to evenly coat the NSCs, and incubate for 2–5 minutes at room temperature.

- **Important:** To maximize transfection efficiency, seeding a single-cell suspension of NSCs prepared with StemPro Accutase reagent is recommended.

4. Observe cells on an inverted microscope to confirm that NSCs are detached; firmly tap the flask to aid in the detachment of NSCs, as necessary.

5. Add 9 mL of StemPro NSC SFM to inactivate the StemPro Accutase reagent.

6. Gently triturate and rinse the flask to generate a single-cell suspension, and transfer the cell suspension into a 15 mL conical tube.

7. Centrifuge the NSC cell suspension at 200 x *g* for 4 minutes.

8. Aspirate the supernatant and resuspend the pellet to a single-cell suspension in 3 mL of StemPro NSC SFM.

9. Perform a total viable cell count with the Invitrogen™ Countess™ II Automated Cell Counter or another method.

10. Dilute with additional StemPro NSC SFM to a final concentration of 150,000 cells/mL.

11. Aspirate the Geltrex matrix from the wells of a precoated 24-well plate.

- **Important:** Proliferating NSC cultures need room to expand during transfection, so plate the recommended starting number of cells (step 12) to achieve 30–60% confluence on the day of transfection.

12. Add 0.5 mL of the NSC suspension in StemPro NSC SFM to plate 75,000 cells/well in the precoated 24-well plate.

13. Return the plate to the incubator and culture at 37°C with 5% CO_2, overnight.

- **Important:** You do not need to change the medium on the day of transfection.

DNA transfection protocol

Perform the following steps, which have been optimized for using Invitrogen™ Lipofectamine™ Stem Transfection Reagent with NSCs:

Step	Tube	Complexation component	Amount per well (24-well plate)
1	Tube 1	Opti-MEM I medium	25 µL
		Lipofectamine Stem reagent	1 µL
2	Tube 2	Opti-MEM I medium	25 µL
		DNA (0.5–5 µg/µL)	500 ng
3	Add tube 2 solution to tube 1, and mix well.		
4	Incubate mixture from step 3 for 10 minutes at room temperature.		
5	Add 50 µL of complex from step 4 to each well; gently swirl plate to ensure even distribution of the complex across the entire well.		
6	Return culture dish to incubator and culture at 37°C with 5% CO_2, overnight.		
7	The following day, overlay an additional 0.5 mL of StemPro NSC SFM per well, if NSCs are going to be transfected for 48 hours.		

Analysis of transfection efficiency

Observe NSCs transfected with a GFP reporter construct at 24 and 48 hours posttransfection by fluorescence microscopy or flow cytometry for endpoint analysis (Figure 1).

Tips and tricks

- The amount of Lipofectamine Stem reagent required for optimal transfection depends on the amount of NSCs plated and the amount of DNA used.
- If cytotoxicity from the DNA preparation is evident, reducing the amount of DNA to 250 ng per well can improve survival while maintaining efficient transfection.
- Using a plasmid with a promoter that is active in human NSCs, such as the EF1α promoter, is critical for assessing transfection efficiency; some promoters such as the cytomegalovirus (CMV) promoter can be transcriptionally silenced in NSCs.

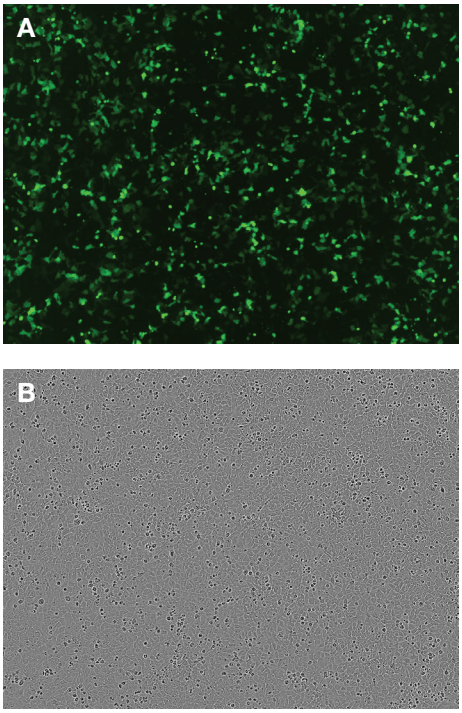

Figure 1. Posttransfection analysis of NSCs. (A) Fluorescence image demonstrating 59% transfection efficiency, and **(B)** bright-field image. NSCs are shown 24 hours after transfection with 500 ng of a 6 kb EF1α-GFP plasmid and 1 µL of Lipofectamine Stem reagent in StemPro NSC SFM on Geltrex matrix.

mRNA transfection protocol

Perform the following steps, which have been optimized for using Lipofectamine Stem reagent with NSCs:

Step	Tube	Complexation component	Amount per well (24-well plate)
1	Tube 1	Opti-MEM I medium	25 µL
		Lipofectamine Stem reagent	1 µL
2	Tube 2	Opti-MEM I medium	25 µL
		mRNA (0.5–5 µg/µL)	250 ng
3	Add tube 2 solution to tube 1, and mix well.		
4	Incubate mixture from step 3 for 10 minutes at room temperature.		
5	Add 50 µL of complex from step 4 to each well; gently swirl plate to ensure even distribution of the complex across the entire well.		
6	Return culture dish to incubator and culture at 37°C with 5% CO_2, overnight.		
7	The following day, overlay an additional 0.5 mL of StemPro NSC SFM per well, if NSCs are going to be transfected for 48 hours.		

Analysis of transfection efficiency

Observe PSCs transfected with a fluorescent mRNA at 24 and 48 hours posttransfection by fluorescence microscopy or flow cytometry for endpoint analysis (Figure 2).

Tips and tricks

- The amount of mRNA required to generate a specific biological readout will vary by user application; Lipofectamine Stem reagent efficiently delivers mRNA into NSCs across a range of dosages.
- Including an independent GFP mRNA (50 ng) in addition to your transcript of interest allows an independent assessment of transfection efficiency.
- If cytotoxicity from the mRNA preparation is evident, reducing the amount of mRNA to 125 ng per well can improve survival while maintaining efficient transfection.
- The method of generation and purification of *in vitro* transcribed (IVT) mRNA can contribute to toxicity as well as translational repression.
  - An anti-reverse cap analog (ARCA) system, included in the Invitrogen™ mMESSAGE mMACHINE™ Kit for *in vitro* transcription, and Invitrogen™ MEGAclear™ columns can be used to eliminate uncapped transcripts and small unincorporated nucleotides that can contribute to cytotoxicity.

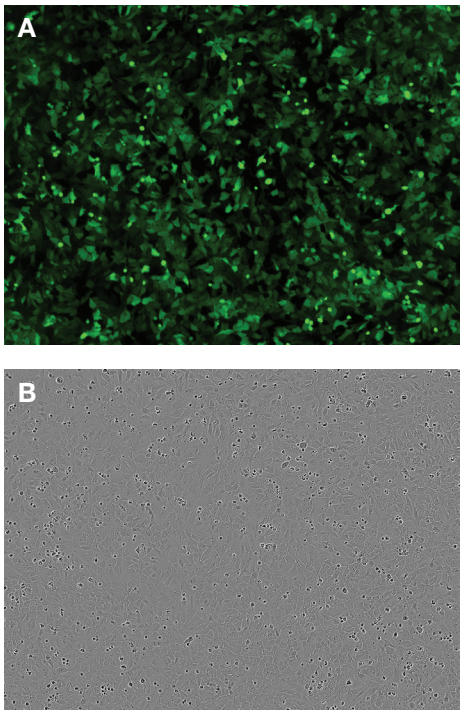

Figure 2. Posttransfection analysis of NSCs. (A) Fluorescence image demonstrating 70% transfection efficiency, and **(B)** bright-field image. iPSC-derived NSCs (NCRM1) are shown 36 hours after transfection with 250 ng of GFP mRNA and 1 µL of Lipofectamine Stem reagent in StemPro NSC SFM on Geltrex matrix.

Ribonucleoprotein (RNP) transfection protocol

RNP complex components:

- Invitrogen™ GeneArt™ Platinum™ Cas9 Nuclease (Cat. No. B25641)
- gRNA (see "Designing and generating gRNA by *in vitro* transcription")

Perform the following steps, which have been optimized for using Lipofectamine Stem reagent with NSCs:

Step	Tube	Complexation component	Amount per well (24-well plate)
1	Tube 1	Opti-MEM I medium	25 µL
		Lipofectamine Stem Reagent	1 µL
2	Tube 2	Opti-MEM I medium	25 µL
		Cas9 nuclease	500 ng
		gRNA (0.1–0.5 µg/µL)	125 ng
3	Add tube 2 solution to tube 1, and mix well.		
4	Incubate mixture from step 3 for 10 minutes at room temperature.		
5	Add 50 µL of complex from step 4 to each well; gently swirl plate to ensure even distribution of the complex across the entire well.		
6	Return culture dish to incubator and culture at 37°C with 5% CO_2, overnight.		
7	The following day, overlay an additional 0.5 mL of StemPro NSC SFM per well, if NSCs are going to be transfected for 48 hours.		

Analysis of transfection efficiency

Observe PSCs transfected with a GFP reporter construct at 24 and 48 hours posttransfection by fluorescence microscopy or flow cytometry, and analyze double-stranded break (DSB) formation using the Invitrogen™ GeneArt™ Genomic Cleavage Detection Kit or a similar assay (Figure 3).

Tips and tricks

- Adding 50 ng of GFP mRNA to the transfection complex along with the RNP complex can provide an independent measure of transfection efficiency.

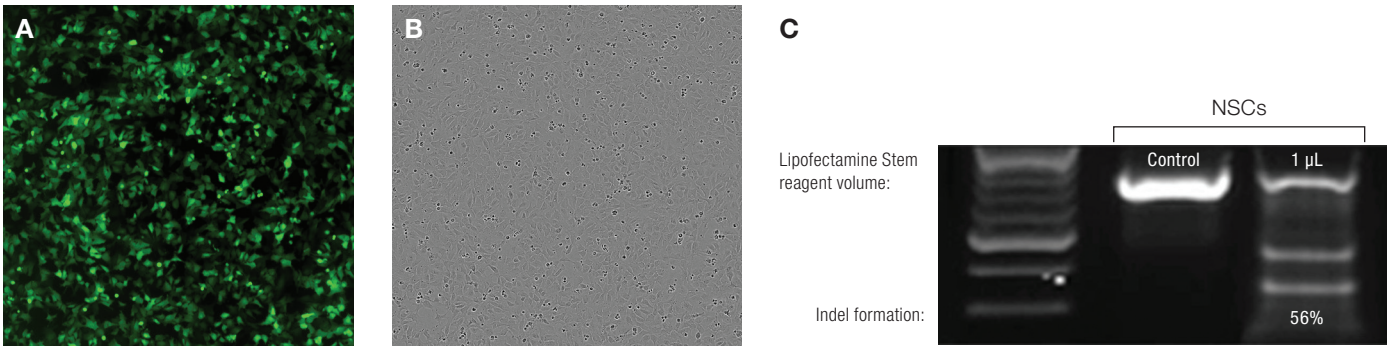

Figure 3. Posttranfection analysis of NSCs. (A) Fluorescence image demonstrating 60% transfection efficiency, and **(B)** bright-field image. iPSC-derived NSCs (NCRM1) are shown 24 hours posttransfection with 500 ng of GeneArt Platinum Cas9 Nuclease, 125 ng of gRNA, 50 ng of GFP mRNA, and 1 µL of Lipofectamine Stem reagent in StemPro NSC SFM on Geltrex matrix. **(C)** Genomic cleavage detection analysis of iPSC-derived NSCs 48 hours posttransfection, demonstrating 56% indel formation within the *EMX1* locus.

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Designing and generating gRNA by *in vitro* transcription

In addition to RNP transfection efficiency, the efficiency of DSB/indel formation at a given locus can depend on gRNA design. Use the Invitrogen™ GeneArt™ CRISPR Search and Design Tool, available at **thermofisher.com/crisprdesign**, to search our database of >600,000 predesigned gRNA sequences specific to every gene in the human genome. These predesigned gRNAs are optimized for gene knockout and typically target the first 3 transcribed exons per gene.

Clone and generate your own gRNA using the Invitrogen™ GeneArt™ Precision gRNA Synthesis Kit (Cat. No. A29377). gRNA concentration can be quantified on the Invitrogen™ Qubit™ 3 Fluorometer (Cat. No. Q33216) coupled with the Invitrogen™ Qubit™ RNA BR Assay Kit (Cat. No. Q10210).

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

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