Non-viral transfection of animal tissue culture cells

Non-viral transfection introduces DNA or RNA into animal tissue culture cells using chemical delivery methods. The approach avoids viral vectors and enables rapid, scalable manipulation of a wide range of cell types to conduct transient expressions, reporter assays, and genetic perturbation screens.

Lipofectamine® reagents (*Alternative A*) are mixtures of cationic lipids (DOSPA) and neutral helper lipids (DOPE) that form liposomes, which fuse with the plasma membrane to release cargo into the cytoplasm. They are broadly effective and widely used in routine applications. FuGENE® (*Alternative B*) and polyethyleneimine (PEI; *Alternative C*) act via electrostatic condensation of nucleic acids into transfectable particles. FuGENE® is serum-tolerant and gentle on cells, while PEI is inexpensive and scalable but more cytotoxic and often less robust. Calcium chloride co-precipitation (*Alternative D*) remains a simple, economical option, though its success depends critically on pH, temperature, and cell type.

Risk assessment

– Work with human-derived material or transgenic cell lines (BSL-2)

▷ Wear gloves, safety glasses, lab coat

□ Collect and dispose waste after inactivation as REGULATED MEDICAL WASTE

Reviewed: Apr 5, 2025

Procedures

›› ***Common provisions***

(1.) Prepare high-quality, endotoxin-free DNA for transfection.

> ***This is why:*** Use endonuclease-negative strains such as DH10B, DH5α, JM109, Stbl2, or TOP10™ to propagate plasmids. If using endA$^+$ strains like HB101 or Stbl3, include an additional wash step during purification.

(2.) One day prior to transfection, seed cells to reach 30–40% confluency on the day of transfection. Use the table below to estimate DNA and medium volumes for each format.

Reagent		**Format**	**Flasks**	**Dishes**			**Plates (per well)**			
			T-175	**15 cm**	**10 cm**	**6 cm**	**6-well**	**12-well**	**24-well**	**96-well**
		Surface area	**175 cm²**	**145 cm²**	**56.7 cm²**	**21.5 cm²**	**9.6 cm²**	**3.5 cm²**	**1.9 cm²**	**1.1 cm²**
Cells to seed		20–30%	8.2×10^6	7.0×10^6	3.0×10^6	1.1×10^6	4.2×10^5	1.8×10^5	8.4×10^4	1.4×10^4
Opti-MEM™		10 µL/cm²	1.8 mL	1.4 mL	550 µL	200 µL	150 µL	100 µL	50 µL	25 µL
DNA	Lipofect.®	250 ng/cm²	44.0 µg	36.0 µg	14.0 µg	5.4 µg	2.4 µg	875 ng	475 ng	275 ng
	FuGENE®	200 ng/cm²	35.0 µg	29.0 µg	11.3 µg	4.3 µg	1.9 µg	700 ng	380 ng	220 ng
	PEI	150 ng/cm²	26.3 µg	21.8 µg	8.5 µg	3.2 µg	1.4 µg	525 ng	285 ng	165 ng
	$CaCl_2$	450 ng/cm²	78.8 µg	65.3 µg	25.5 µg	9.7 µg	4.3 µg	1.58 µg	855 ng	495 ng

> ***Critical:*** Adjustments may be necessary based on transfection method, cell type, or well geometry. If your format is not listed, calculate DNA and medium volume based on surface area and initial seeding density.

(3.) *Critical:* Consult the manufacturer's protocols for suspension, primary, or difficult-to-transfect cells.

> ***Hint:*** Lipofectamine® 3000 is formulated for use with stem cells and suspension lines, but may require different reagent volumes or co-factors such as P3000 reagent. Lipofectamine® 3000 uses similar amounts of DNA as Lipofectamine® 2000.

A › *Transfection with Lipofectamine® 2000*

- ☐ Reduced-serum medium (Opti-MEM™)
- ☐ Lipofectamine® 2000

(1.) Dilute endotoxin-free DNA in Opti-MEM™. Use the volumes given in the planning table above.

(2.) In a separate tube, dilute 3.6 µL Lipofectamine® 2000 per microgram DNA with the same volume of Opti-MEM™ as the DNA solution. Mix gently.

> ***Critical:*** Lipofectamine® amounts should be optimized for each cell line. Adjust by increasing or decreasing 40% to balance efficiency and toxicity. Do not change the Opti-MEM™ volume.

> ***This is why:*** Serum inhibits complex formation by binding cationic lipids or interfering with particle stability. Therefore, Lipofectamine–DNA complexes are preformed in serum-free medium such as Opti-MEM™. Once complexes are added, serum can usually be tolerated.

(3.) Combine equal volumes of DNA and Lipofectamine® 2000 mixtures. Gently pipette or vortex briefly. Incubate at room temperature for 5–20 min, but not longer. ⏲ 20 min

(4.) In the meantime, reduce or replace the cell culture medium volume to 50%.

(5.) Dispense the DNA–lipid complexes dropwise to each well. Gently rock to distribute; avoid swirling.

(6.) *Optional:* After 6–24 h, replace the medium.

[YH98]

B › *Transfection with FuGENE®*

(1.) Dilute DNA in Opti-MEM™ as above. Add FuGENE® at 3 µL per microgram DNA. Vortex briefly.

> ***Note:*** FuGENE® is serum-compatible and often requires no medium change post-transfection.

(2.) Incubate DNA–FuGENE® complexes at room temperature for 10–15 min. ⏲ 15 min

(3.) Apply complexes dropwise to cells in standard culture medium. Gently rock to distribute.

C › *Transfection with polyethyleneimine (PEI)*

- ☐ Reduced-serum medium (Opti-MEM™)
- ☐ 1 g/L Polyethyleneimine, 500 µL ⟨*R*⟩

(1.) Dilute DNA in Opti-MEM™ as above.

(2.) In a separate tube, dilute 3.0 µL polyethyleneimine (PEI) per microgram DNA with the same volume of Opti-MEM™ as the DNA solution.

(3.) Combine. Vortex briefly. Incubate DNA–PEI complexes for 10–30 min at room temperature. ⏲ 10 min

(4.) Dilute 6-fold with Opti-MEM™. Apply dropwise to cells. Rock gently; avoid swirling.

> ***Hint:*** For sensitive cell lines, gently pipette the solution down the side of the well and not on top of the cells, so as not to disrupt the adherent cells.

(5.) *Optional:* After 6–24 h, replace the medium.

[BLZ+95]

D › *Transfection with calcium chloride*

- ☐ 2 × HEPES-buffered saline, 1 mL ⟨*R*⟩
- ☐ 1 M Calcium chloride

(1.) Seed cells at 20–30% confluency already 4–6 h before transfection.

(2.) Dilute DNA in water and supplement $CaCl_2$ to a final concentration of 250 mM.

> ***Note:*** Supercoiled DNA (plasmids) yields best results.

(3.) *Critical:* Drop by drop, add an equal volume of 2 × HEPES-buffered saline. Vortex after each drop. Do not let the calcium complex precipitate!

(4.) Incubate at room temperature for 15 min. 15 min

(5.) Apply dropwise to cells. Rock gently; avoid swirling.

> ***Critical:*** Calcium-mediated uptake depends on temperature and pH. Keep medium pH stable and avoid cell overgrowth.

(6.) After 6–12 h, remove the medium. Wash cells with PBS and feed fresh medium.

[KCO03]

Troubleshooting

Transfection with Lipofectamine® 2000

In Step 6:

– High cytotoxicity with low transfection efficiency

- Replace or supplement serum-free medium with full medium after 3–9 h.
- Reduce Lipofectamine volume by 40%. Excessive cationic lipid damages membranes without improving DNA delivery.
- Ensure cells are 30–40% confluent at transfection. Over-confluent cells are less permissive to lipoplex uptake.

Recipes

Polyethyleneimine (PEI), pH 7.0, 1 g/L

Amount	Ingredient	Stock	Final
100 mg	Polyethyleneimine (PEI), linear, transfection-grade [26913-06-4]	25 000 g/mol	
To 100 mL	Water, reagent-grade		

Bring the stirred solution to neutral pH. Be patient, this may take hours! Recheck pH after 15 min to ensure it has not drifted. Filter-sterilize through a 0.22 µm polyethersulfone (PES) membrane to remove undissolved particles. Dispense into 500 µL aliquots. Store at −80 °C. ***Note:*** Stable for two months at 4 °C. Purchase PEI from Polysciences (Cat. no. 23966).

1 g/L Polyethyleneimine (PEI)

pH 7.0

Skin sensitization; Eye irritation; Hazardous to the aquatic environment

Date: Sign: R0131

HEPES-buffered saline, pH 7.1, 2 ×

Amount	Ingredient	Stock	Final
25 mL	HEPES, pH 7.5 R0024	1 M	50 mM
28 mL	NaCl R0046	5 M	280 mM
0.2 g	$Na_2HPO_4 \cdot 7\ H_2O$ [7782-85-6]	268.07 g/mol	1.5 mM
5.4 mL	Glucose, sterile-filtered R0021	20%	12 mM
To 500 mL	Water, reagent-grade		

Filter sterilize. Stable for 1 year at room temperature or as frozen aliquots.

2 × HEPES-buffered saline

50 mM HEPES, 280 mM NaCl, 1.5 mM Na_2HPO_4, 12 mM Glucose, pH 7.1

Expiry: Sign: R0132

List of references

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R.E. Kingston, C.A. Chen, and H. Okayama, *Curr. Protocol. Cell BIol.* **Chapter 20** Unit 20.3 (2003).

J. Yang and L. Huang, *Gene Ther.* **5**(3), 380—387 (1998).

Change log

2017-05-15	Moritz Pott	Initial calcium chloride protocol; Daniel Summerer lab.
2020-05-20	Yael David	Initial Lipofectamine® protocol; Tom Muir lab.
⋮	⋮	⋮
2023-02-09	Benjamin C. Buchmuller	Added FuGENE® and PEI protocols.
2023-04-04	Benjamin C. Buchmuller	Updated Lipofectamine® volumes.
2025-04-05	Benjamin C. Buchmuller	Harmonized provisions. Updated safety information for PEI.