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 cationic lipid formulations (the original Lipofectamine combines DOSPA with the neutral helper lipid DOPE) that form lipid–DNA complexes, which cells take up mainly by endocytosis. They are broadly effective and widely used in routine applications. FuGENE® (*Alternative B*) is a nonliposomal mixture of lipids and other components; polyethyleneimine (PEI; *Alternative C*) condenses nucleic acids electrostatically 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

□ Dispose waste after inactivation as REGULATED MEDICAL WASTE



Reviewed: Nov 4, 2026

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 before transfection, seed cells as in the table below, at about 20–30% confluency, so that they reach 70–90% on the day of analysis. Transfect one to three days before analysis, depending on the readout. 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 cm^2	145 cm^2	56.7 cm^2	21.5 cm^2	9.6 cm^2	3.5 cm^2	1.9 cm^2	0.32 cm^2
Cells to seed		5×10^4/cm^2	9×10^6	7×10^6	3×10^6	1×10^6	5×10^5	1.8×10^5	1×10^5	1.5×10^4
Opti-MEM™		13 µL/cm^2	2.3 mL	1.9 mL	750 µL	280 µL	125 µL	45 µL	25 µL	5 µL
DNA	Lipofect.®	250 ng/cm^2	40 µg	35 µg	14 µg	5 µg	2.5 µg	900 ng	500 ng	80 ng
	FuGENE®	300 ng/cm^2	50 µg	40 µg	17 µg	6 µg	3 µg	1 µg	500 ng	100 ng
	PEI	300 ng/cm^2	50 µg	40 µg	17 µg	6 µg	3 µg	1 µg	500 ng	100 ng
	$CaCl_2$	450 ng/cm^2	80 µg	65 µg	25 µg	10 µg	4 µg	1.5 µg	850 ng	150 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 hard-to-transfect cells, 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*

- ☐ Transfer DNA, endotoxin-free plasmid; supercoiled preferred
- ☐ Lipofectamine® 2000 Transfection Reagent
- ☐ Opti-MEM™ I Reduced Serum Medium

(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. ⏲ 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 6–12 h, replace the medium.

🔗 [The13]

B › *Transfection with FuGENE®*

- ☐ Transfer DNA, endotoxin-free plasmid
- ☐ FuGENE® 6 Transfection Reagent
- ☐ Opti-MEM™ I Reduced Serum Medium

(1.) Dilute FuGENE® at 3 µL per microgram DNA in Opti-MEM™ without touching the tube wall, incubate for 5 min, then add the DNA. Mix gently.

> ***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.

🔗 [Pro25]

C › *Transfection with polyethyleneimine (PEI)*

- ☐ Transfer DNA, endotoxin-free plasmid
- ☐ 1 g/L PEI, pH 7.0 ◇ R0131
- ☐ Opti-MEM™ I Reduced Serum Medium

(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 30 min at room temperature. ⏲ 30 min

(4.) 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*

- ☐ Transfer DNA, endotoxin-free plasmid; supercoiled preferred
- ☐ 2 × HEPES-buffered saline, pH 7.1 R0132
- ☐ PBS, pH 7.5 (at 1 ×) R0037
- ☐ 1 M $CaCl_2$ R0012

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

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

(2.) *Critical:* Place an equal volume of 2 × HEPES-buffered saline in a tube. While bubbling air through it with a pipettor, add the DNA–$CaCl_2$ solution drop by drop. Vortex for 5 s. Avoid a coarse precipitate; a fine precipitate is visible only under the microscope.

(3.) Incubate at room temperature for 20 min. 20 min

(4.) 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.

(5.) 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
  - Reduce Lipofectamine volume by 40%.

Materials

Transfection with Lipofectamine® 2000

- ☐ **Transfer DNA**, endotoxin-free plasmid; supercoiled preferred
- ☐ **Opti-MEM™ I Reduced Serum Medium**
  Thermo Fisher Scientific, 31985070
- ☐ **Lipofectamine® 2000 Transfection Reagent**
  Thermo Fisher Scientific, 11668019

Transfection with FuGENE®

- ☐ **Transfer DNA**, endotoxin-free plasmid
- ☐ **Opti-MEM™ I Reduced Serum Medium**
  Thermo Fisher Scientific, 31985070
- ☐ **FuGENE® 6 Transfection Reagent**
  Promega, E2691

Transfection with polyethyleneimine (PEI)

- ☐ **Transfer DNA**, endotoxin-free plasmid
- ☐ **Opti-MEM™ I Reduced Serum Medium**
  Thermo Fisher Scientific, 31985070
- ☐ **Polyethyleneimine (PEI)**, 1 g/L, pH 7.0 ⟋ R0131
  Stable for 9 months at 4 °C.

Transfection with calcium chloride

- ☐ **Transfer DNA**, endotoxin-free plasmid; supercoiled preferred
- ☐ **Phosphate-buffered saline (PBS)**, pH 7.5 (at 1 ×) ⟋ R0037
- ☐ **HEPES-buffered saline**, 2 ×, pH 7.1 ⟋ R0132
  Store at −20 °C.
- ☐ **Calcium chloride ($CaCl_2$)**, 1 M ⟋ R0012

Recipes

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

Amount	Ingredient	Stock	Final
100 mg	Polyethyleneimine (PEI), linear, transfection-grade [9002-98-6]	25 000 g/mol	
To 100 mL	Water, reagent-grade		

Add hydrochloric acid dropwise to the stirred suspension until it clears. The free base does not dissolve at neutral pH; be patient, this may take hours! Bring the stirred solution to pH 7.0 with sodium hydroxide, a few drops at a time. 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. Stable for 9 months at 4 °C. Polyethyleneimine from Polysciences (23966). ***Note:*** PEI MAX (linear polyethyleneimine hydrochloride, 40 kDa; Polysciences, Cat. no. 24765) is an alternative. Being a salt, it dissolves in water directly and needs no acid step.

1 g/L Polyethyleneimine (PEI)
pH 7.0



No GHS hazards at the indicated concentrations

☐ Hold for EHS review before disposal

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
0.106 g	Sodium phosphate dibasic (Na_2HPO_4)	[7558-79-4]	141.96 g/mol	1.5 mM
5.4 mL	Glucose, sterile-filtered	R0021	20%	12 mM
To 500 mL	Water, reagent-grade			

Adjust to pH 7.1 (7.05 to 7.12) at room temperature before making up to volume. Filter sterilize. Store at −20 °C. ***Critical:*** The pH is extremely critical for a successful transfection; even 0.1 pH units deviation reduces efficiency!

2 × HEPES-buffered saline

25 mM HEPES, 140 mM NaCl, 750 µM Na_2HPO_4, 6 mM Glucose, pH 7.1

No GHS hazards at the indicated concentrations

- ☐ Dilute below the discharge limit
- ☐ Flush to drain with excess water

Date: Sign: R0132

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Change log

2026-06-11 **Moritz Pott, Yael David, Benjamin C. Buchmuller** Version 2, 2017 to 2026. Calcium chloride protocol from the Daniel Summerer lab; Lipofectamine® protocol from the Tom Muir lab; adapted as an SOP in 2023, then extended with FuGENE® and PEI. HBS moved from the heptahydrate to anhydrous sodium phosphate dibasic; PEI safety information updated.

2026-11-04 **Benjamin C. Buchmuller** Version 3. Transfection amounts change: DNA per well, the confluency at transfection, and the FuGENE and PEI ratios. The Opti-MEM volume per tube follows Thermo Fisher's own per-well volumes at 13 µL/cm², up from ten; the seeding density is one figure, 5×10^4/cm², where the row had drifted between 4.4 and 5.3; and the DNA masses are rounded to planning figures. Credits: Thermo Fisher (MAN0007824) and Promega (TM350) for the lipid handling, Graham and van der Eb (1973) for calcium phosphate, Kingston et al. (2003) and Longo et al. (2013) for the PEI ratios. Also corrected the PEI CAS number, added PEI MAX, and corrected the HBS pH and storage, the PEI storage, and the order of addition for FuGENE and calcium phosphate. Linear PEI is dissolved with acid before neutralising.

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