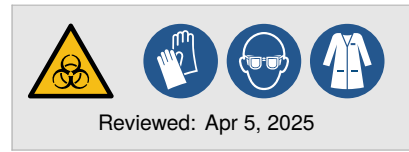


Non-viral transfection of animal tissue culture cells

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.

This is a bench card. Full protocol available online.



Procedures

>> Common provisions

- (1.) Prepare high-quality, endotoxin-free DNA for transfection. 📖
- (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 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	1.1 cm ²				
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/cm ²	1.8 mL	1.4 mL	550 µL	200 µL	150 µL	100 µL	50 µL	25 µL				
DNA	Lipofect.®	250 ng/cm ²	44.0 µg	36.0 µg	14.0 µg	5.4 µg	2.4 µg	875 ng	475 ng	275 ng			
	FuGENE®	200 ng/cm ²	35.0 µg	29.0 µg	11.3 µg	4.3 µg	1.9 µg	700 ng	380 ng	220 ng			
	PEI	150 ng/cm ²	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/cm ²	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. 📖



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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. 
- (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. 

B > **Transfection with FuGENE®**

- (1.) Dilute DNA in Opti-MEM™ as above. Add FuGENE® at 3 µL per microgram DNA. Vortex briefly. 
- (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™)  R0131 1 g/L Polyethyleneimine, 500 µL

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

D > **Transfection with calcium chloride**

 R0132 2 × HEPES-buffered saline, 1 mL  R0012 1 M Calcium chloride

- (1.) Seed cells at 20–30% confluency already 4–6 h before transfection.
- (2.) Dilute DNA in water and supplement CaCl₂ to a final concentration of 250 mM. 
- (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

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(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.

List of references

[🔗](#) Recipe (available online) [🔗](#) Troubleshooting (available online) [📄](#) Notes (available online)

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