

Lipofectamine® 3000 Reagent Protocol

Protocol Outline

- Plate cells so they will be 70–90% confluent at the time of transfection.
- Prepare plasmid DNA-lipid complexes (recommend 2 doses of lipid).
- Add DNA-lipid complexes to cells.

Transfection Amounts

Component	96-well	24-well	6-well
DNA per well	100 ng	500 ng	2500 ng
P3000™ Reagent per well	0.2 µL	1 µL	5 µL
Lipofectamine® 3000 Reagent per well	0.15 and 0.3 µL	0.75 and 1.5 µL	3.75 and 7.5 µL

Transfection of siRNA

To transfect cells with siRNA, follow the protocol as described for DNA but **do not** add P3000™ Reagent when diluting the siRNA (step 3).

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Lipofectamine® 3000 Reagent Protocol


invitrogen[™]
by life technologies[®]

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	Catalog Numbers	Size:
 Package Contents	L3000001	0.1 mL
	L3000008	0.75 mL
	L3000015	1.5 mL
	L3000075	5 × 1.5 mL
	L3000150	15 mL


 Storage Conditions	▪ Store at 4°C (do not freeze).
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 Required Materials	▪ Plasmid DNA (0.5–5 µg/µL stock) ▪ Opti-MEM® Reduced Serum Medium ▪ Microcentrifuge tubes
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 Timing	Preparation: 10 minutes Incubation: 5 minutes Final Incubation: 1–3 days
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 Selection Guide	Lipofectamine® Reagents Go online to view related products.
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 Product Description	▪ Lipofectamine® 3000 Reagent is a proprietary formulation for transfecting nucleic acids into a wide range of eukaryotic cells and especially designed for hard to transfect cells
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 Important Guidelines	▪ Make DNA-Lipofectamine® 3000 complexes in serum-free medium such as Opti-MEM® Reduced Serum Medium and add directly to cells in culture medium, in the presence or absence of serum/antibiotic.
	▪ It is not necessary to remove complexes or change/add medium after transfection.
	▪ The amount of Lipofectamine® 3000 Reagent for successful transfection varies. Start any new transfection by testing the recommended two concentrations of Lipofectamine® 3000 Reagent to determine an optimum amount.

 Online Resources	Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support .
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Lipofectamine® 3000 Transfection Reagent Protocol

Transfect cells according to the following table. Use the indicated volume of DNA and P3000™ Reagent with each of the two volumes of Lipofectamine® 3000 (when performing optimization). Each reaction mix volume is for one well and accounts for pipetting variations. Scale volumes proportionally for additional wells.

Timeline		Steps	Procedure Details (Two Reaction Optimization)			
Day 0	1	Seed cells to be 70–90% confluent at transfection	Component	96-well	24-well	6-well
	2	Dilute Lipofectamine® 3000 Reagent in Opti-MEM® Medium (2 tubes) – Mix well	Adherent cells	1–4 × 10 ⁴	0.5–2 × 10 ⁵	0.25–1 × 10 ⁶
	3	Prepare master mix of DNA by diluting DNA in Opti-MEM® Medium, then add P3000™ Reagent – Mix well	Opti-MEM® Medium	5 µL × 2	25 µL × 2	125 µL × 2
Day 1	4	Add Diluted DNA to each tube of Diluted Lipofectamine® 3000 Reagent (1:1 ratio)	Lipofectamine® 3000 Reagent	0.15 and 0.3 µL	0.75 and 1.5 µL	3.75 and 7.5 µL
	5	Incubate	Opti-MEM® Medium	10 µL	50 µL	250 µL
	6	Add DNA-lipid complex to cells	DNA (0.5–5 µg/µL)	0.2 µg	1 µg	5 µg
	7	Visualize/analyze transfected cells	P3000™ Reagent (2 µL/µg DNA)	0.4 µL	2 µL	10 µL
			Diluted DNA (with P3000™ Reagent)	5 µL	25 µL	125 µL
Day 2–4			Diluted Lipofectamine® 3000 Reagent	5 µL	25 µL	125 µL
			Incubate for 5 minutes at room temperature.			
			Component (per well)	96-well	24-well	6-well
			DNA-lipid complex	10 µL	50 µL	250 µL
			DNA amount	100 ng	500 ng	2500 ng
			P3000™ Reagent	0.2 µL	1 µL	5 µL
			Lipofectamine® 3000 Reagent used	0.15 and 0.3 µL	0.75 and 1.5 µL	3.75 and 7.5 µL
			Incubate cells for 2–4 days at 37°C. Then, analyze transfected cells.			