Lipofectamine® 3000 Reagent Protocol

Protocol Outline

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



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	Catalog Numbers	Size:
	L3000001	0.1 mL
Daalrana	L3000008	0.75 mL
Package	L3000015	1.5 mL
Contents	L3000075	$5 \times 1.5 \text{ mL}$
	L3000150	15 mL

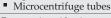


Storage Conditions

■ Store at 4°C (do not freeze).



Required Materials Plasmid DNA (0.5–5 μg/μL stock)
 Opti-MEM® Reduced Serum Medium





Timing

Preparation: 10 minutes Incubation: 5 minutes Final Incubation: 1–3 days



Selection Guide Lipofectamine® Reagents
Go online to view related products.



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



Important Guidelines 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 presessary to remove complexes or change/add

■ Make DNA-Lipofectamine® 3000 complexes in serum-free

- 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 Reagent Protocol

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.

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	4	1 4	Seed cells to be 70-90% confluent at transfection	Component	96-well	24-well	6-well	
	1			Adherent cells	$1-4 \times 10^4$	$0.5-2 \times 10^5$	$0.25-1 \times 10^6$	
Day 1		Diluted Lipofectamine® 3000	Dilute Lipofectamine® 3000 Reagent in Opti-MEM® Medium (2 tubes) – Mix well	Opti-MEM® Medium	5 μL × 2	25 μL × 2	125 μL × 2	
	2	Vortex 2-3 sec		Lipofectamine® 3000 Reagent	0.15 and 0.3 μL	0.75 and 1.5 μL	3.75 and 7.5 μL	
		3	Prepare master mix of DNA by diluting DNA in Opti- MEM® Medium, then add P3000™ Reagent – Mix well	Opti-MEM® Medium	10 μL	50 μL	250 μL	
	3			DNA (0.5–5 $\mu g/\mu L$)	0.2 μg	1 μg	5 μg	
				P3000™ Reagent (2 µL/µg DNA)	0.4 μL	2 μL	10 μL	
	1		Add Diluted DNA to each tube of Diluted	Diluted DNA (with P3000 [™] Reagent)	5 μL	25 μL	125 μL	
	4	Lipofectamine® 3000 Reagent (1:1 ratio)	Diluted Lipofectamine® 3000 Reagent	5 μL	25 μL	125 μL		
	5	5	Incubate	Incubate for 5 minutes at room temperature.				
		6 10	Add DNA-lipid complex to cells	Component (per well)	96-well	24-well	6-well	
				DNA-lipid complex	10 μL	50 μL	250 μL	
	6			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	
Day 2-4	7		Visualize/analyze transfected cells	Incubate cells for 2–4 days at 37°C. Then, analyze transfected cells.				