invitrogen

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Package Contents

Catalog Numbers	Size:
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).

Required Materials

- Plasmid DNA (0.5–5 µg/µL stock)
- Opti-MEM™ Reduced Serum Medium
- Microcentrifuge tubes

Timing

Preparation: 10 minutes
Incubation: 10–15 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

- 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 **thermofisher.com/support**

For Research Use Only. Not for use in diagnostic procedures.

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

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)			
				Component	96-well	24-well	6-well
Day 0	1		**Seed cells to be 70–90% confluent at transfection**	Adherent cells	1–4 × 10^4	0.5–2 × 10^5	0.25–1 × 10^6
Day 1	2	 Vortex 2–3 sec	**Dilute Lipofectamine™ 3000 Reagent in Opti-MEM™ Medium (2 tubes) – Mix well**	Opti-MEM™ Medium	5 µL × 2	25 µL × 2	125 µL × 2
				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
				DNA (0.5–5 µg/µL)	0.2 µg	1 µg	5 µg
				P3000™ Reagent (2 µL/µg DNA)	0.4 µL	2 µL	10 µL
	4		**Add Diluted DNA to each tube of Diluted Lipofectamine™ 3000 Reagent (1:1 ratio)**	Diluted DNA (with P3000™ Reagent)	5 µL	25 µL	125 µL
				Diluted Lipofectamine™ 3000 Reagent	5 µL	25 µL	125 µL
	5		**Incubate**	Incubate for 10–15 minutes at room temperature.			
				Component (per well)	96-well	24-well	6-well
	6		**Add DNA-lipid complex to cells**	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
Day 2–4	7		**Visualize/analyze transfected cells**	Incubate cells for 2–4 days at 37°C. Then, analyze transfected cells.			