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
Enhancer™ Reagent per well	0.2 µg	1 µg	5 µg
Lipofectamine® 3000 Reagent per well	0.15 and 0.3 µL	0.75 and 1.5 µL	3.75 and 7.5 µL

See the table inside of the foldout to view a typical culturing procedure along with a diagram.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

Important Licensing Information

These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Disclaimer

LIFE TECHNOLOGIES CORPORATION AND/OR ITS AFFILIATE(S) DISCLAIM ALL WARRANTIES WITH RESPECT TO THIS DOCUMENT, EXPRESSED OR IMPLIED, INCLUDING BUT NOT LIMITED TO THOSE OF MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE, OR NON-INFRINGEMENT. TO THE EXTENT ALLOWED BY LAW, IN NO EVENT SHALL LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) BE LIABLE, WHETHER IN CONTRACT, TORT, WARRANTY, OR UNDER ANY STATUTE OR ON ANY OTHER BASIS FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING BUT NOT LIMITED TO THE USE THEREOF.

life technologies™

For support, visit www.lifetechnologies.com/support.

Lipofectamine® 3000 Reagent Protocol

invitrogen by life technologies™

	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).

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

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
- 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 required 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.

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

Lipofectamine® 3000 Transfection Reagents Protocol

Transfect cells according to the following table. Use the indicated volume of DNA and P3000™ Reagent with each of the 2 volumes of Lipofectamine® 3000. **Each reaction mix is given per well and accounts for pipetting variations.**

Timeline			Steps	Procedure Details			
				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
	2	 Vortex 2–3 sec	**Dilute Lipofectamine® 3000 Reagent in Opti-MEM® Medium – 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
Day 1	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 5 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
				Enhancer amount	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.			