invitrogen
by life technologies

Lipofectamine® 2000 Reagent

Cat. nos.	11668-030	**Size:** 0.3 mL	**Store at 4°C (do not freeze)**
	11668-027	0.75 mL	
	11668-019	1.5 mL	
	11668-500	15 mL	

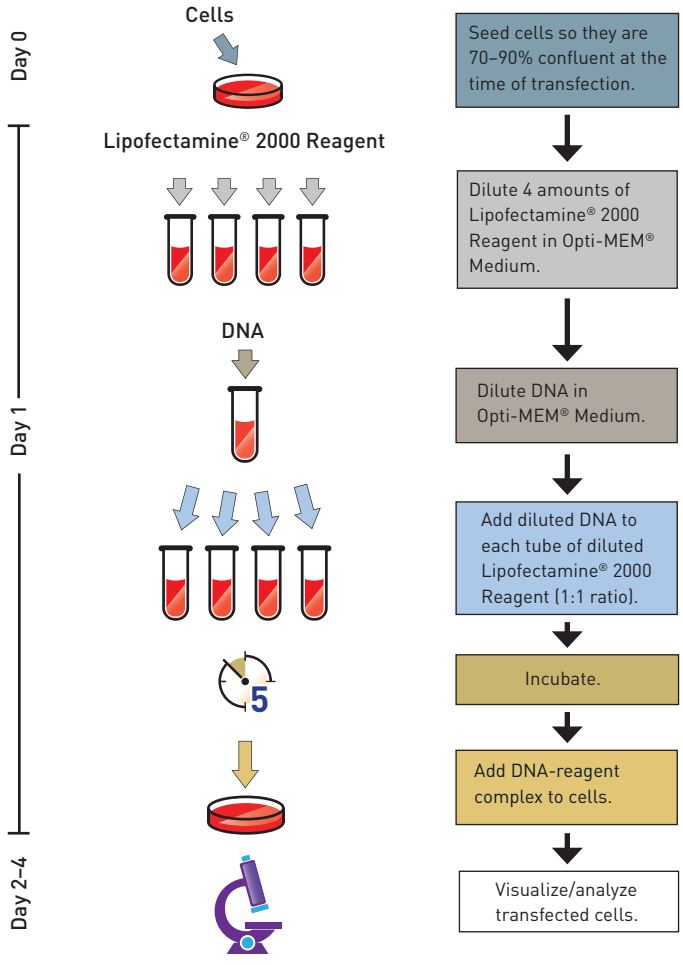
Pub. Part No. 11668.2k.pps **Pub. No.** MAN0000995 **Rev. Date** 20 July 2012

Description

- Lipofectamine® 2000 Transfection Reagent is a proprietary formulation for transfecting nucleic acids (DNA, RNA, and mRNA) into a wide range of eukaryotic cells.
- Nucleic acid-Lipofectamine® 2000 complexes can be added 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.

Important Guidelines for Transfection

- Use Opti-MEM® I Reduced Serum Medium (Cat. no. 31985-062) to dilute Lipofectamine® 2000 Transfection Reagent and nucleic acids.
- The amount of Lipofectamine® 2000 Reagent required for successful transfection varies depending on the cell type and passage number. Start any new transfection by testing the recommended four concentrations of Lipofectamine® 2000 Reagent to determine an optimum amount.
- Prepare high-quality plasmid DNA at 0.5–5 µg/µL in deionized water or TE buffer. A GFP (green fluorescent protein) plasmid can be used to determine transfection efficiency.
- For additional information, and protocols on transfecting suspension cells refer to the manual at www.lifetechnologies.com/transfection.


Day 0
Day 1
Day 2–4
Cells
Lipofectamine® 2000 Reagent
DNA
5
Seed cells so they are 70–90% confluent at the time of transfection.
Dilute 4 amounts of Lipofectamine® 2000 Reagent in Opti-MEM® Medium.
Dilute DNA in Opti-MEM® Medium.
Add diluted DNA to each tube of diluted Lipofectamine® 2000 Reagent (1:1 ratio).
Incubate.
Add DNA-reagent complex to cells.
Visualize/analyze transfected cells.

Plasmid Transfection

Transfect cells according to the following table. The transfection is designed for 1 DNA amount combined with 4 different amounts of Lipofectamine® 2000. For each lipid reagent amount, the prepared mix is enough to have triplicates (96-well), duplicates (24-well), and single well (6-well) transfections, and account for pipetting variations.

Component	96-well	24-well	6-well
Adherent cells	1–4 x 10^4	0.5–2 x 10^5	0.25–1 x 10^6
Opti-MEM® Medium	25 µL x 4	50 µL x 4	150 µL x 4
Lipofectamine® 2000 Reagent	1, 1.5, 2, 2.5 µL	2, 3, 4, 5 µL	6, 9, 12, 15 µL
Opti-MEM® Medium	125 µL	250 µL	700 µL
DNA (0.5–5 µg/µL)	2.5 µg	5 µg	14 µg
Diluted DNA	25 µL	50 µL	150 µL
Diluted Lipofectamine® 2000 Reagent	25 µL	50 µL	150 µL
Incubate for 5 minutes at room temperature			
DNA-reagent complex/well	10 µL	50 µL	250 µL
Incubate cells for 1–3 days at 37°C			

The following table shows the amounts of DNA and Lipofectamine® 2000 Reagent per well used in each transfection reaction. For additional information on scaling your transfection reaction, see page 4.

Amount	96-well	24-well	6-well
DNA/well	100 ng	500 ng	2500 ng
Lipofectamine® 2000 Reagent/well	0.2–0.5 µL	1–2.5 µL	5–12.5 µL

Scaling Up or Down Transfections

Use the following table to scale the volumes for your transfection experiment.

Culture Vessel	Multiplication factor[1]	Shared reagents		DNA transfection		RNAi transfection	
		Vol. growth medium	Opti-MEM/ medium vol. for complex	DNA (µg)	Lipid reagent[2] (µL)	RNA (pmol)	Lipid reagent[2] (µL)
96-well	0.17	100 µL	2 × 5 µL	0.1	0.2–0.5	3	0.3
48-well	0.50	250 µL	2 × 12.5 µL	0.25	0.5–1.3	7.5	0.75
24-well	**1.00**	**500 µL**	**2 × 25 µL**	**0.5**	**1–2.5**	**15**	**1.5**
12-well	2.00	1 mL	2 × 50 µL	1	2–5	30	3
6-well	5.00	2 mL	2 × 100 µL	2.5	5–12.5	75	7.5
60-mm	11.05	5 mL	2 × 250 µL	5.5–11	11–28	166	17
10-cm	28.95	10 mL	2 × 500 µL	14–28	29–73	434	43
T75	39.47	15 mL	2 × 750 µL	20–40	39–100	592	59
T175	92.11	35 mL	2 × 1.75 mL	46–90	92–230	1382	138

[1] After determining the optimum reagent amount, use the multiplication factor to determine the reagent amount needed for your new plate format.
[2] Optimum amount needed is determined from the protocol (see pages 2–3).

Co-Transfection of Plasmid DNA and siRNA

Transfect plasmid DNA and siRNA at the same time using Lipofectamine® 2000 Reagent by adding 30 pmol (~0.6 µg) of siRNA per 1 µg of DNA.

mRNA Transfection

mRNA can be transfected in a 24-well plate by using Lipofectamine® 2000 Reagent by adding 0.5–1 µg of mRNA per well.