

Lipofectamine® 2000 Reagent



Package Contents

Catalog Number	Size
11668-030	0.3 mL vial
11668-027	0.75 mL vial
11668-019	1.5 mL vial
11668-500	15 mL vial



Storage Conditions

Store at 4°C (do not freeze).



Required Materials

- Plasmid DNA (0.5–5 µg/µL stock)
- Opti-MEM® Reduced Serum Medium
- Eppendorf 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® 2000 Reagent is a proprietary formulation for transfecting nucleic acids into a wide range of eukaryotic cells.



Important Guidelines

- DNA-Lipofectamine® 2000 complexes must be made in serum-free medium such as Opti-MEM® Reduced Serum Medium and 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.
- 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.



Online Resources

Visit our [product page](#) for additional information and protocols. For support, visit www.lifetechnologies.com/support.



Protocol Outline

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

Lipofectamine® 2000 DNA Transfection Reagent Protocol

i See page 2 to view a typical DNA transfection procedure.

Component	96-well	24-well	6-well
Final DNA per well	100 ng	500 ng	2500 ng
Final Lipofectamine® 2000 Reagent per well	0.2–0.5 µL	1.0–2.5 µL	5.0–12.5 µL

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 using Lipofectamine® 2000 Reagent by adding 0.5–1 µg of mRNA per well.

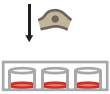




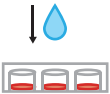

i **Photograph of Expected Results**

i **Scaling Up or Down Transfections**

i **Limited Product Warranty and Disclaimer Details**

Lipofectamine® 2000 DNA Transfection Reagent Protocol

Transfect cells according to the following chart. Volumes are given on a per-well basis. **Each reaction mix is sufficient for triplicate (96-well), duplicate (24-well), and single well (6-well) transfections, and accounts for pipetting variations.** Adjust the amounts of components according to your tissue culture format. For additional information on scaling your transfection reaction, see page 1.

Timeline		Steps	Procedure Details				
Day 0	1		Seed cells to be 70–90% confluent at transfection	Component	96-well	24-well	6-well
	2		Dilute four amounts of Lipofectamine® Reagent in Opti-MEM® Medium	Adherent cells	1–4 × 10 ⁴	0.5–2 × 10 ⁵	0.25–1 × 10 ⁶
Day 1	3		Dilute DNA in Opti-MEM® Medium	Opti-MEM® Medium	25 µL × 4	50 µL × 4	150 µL × 4
	4		Add diluted DNA to diluted Lipofectamine® 2000 Reagent (1:1 ratio)	Lipofectamine® 2000 Reagent	i 1, 1.5, 2, 2.5 µL	i 2, 3, 4, 5 µL	i 6, 9, 12, 15 µL
	5		Incubate	Opti-MEM® Medium	125 µL	250 µL	700 µL
	6		Add DNA-lipid complex to cells	DNA (0.5–5 µg/µL)	2.5 µg	5 µg	14 µg
	7		Visualize/analyze transfected cells	Diluted DNA Total	25 µL	50 µL	150 µL
Day 2–4				Diluted Lipofectamine® 2000 Reagent	25 µL	50 µL	150 µL
				Incubate for 5 minutes at room temperature.			
				Component	96-well	24-well	6-well
				DNA-lipid complex per well	10 µL	50 µL	250 µL
			Final DNA used per well	100 ng	500 ng	2500 ng	
			Final Lipofectamine® 2000 Reagent used per well	0.2–0.5 µL	1.0–2.5 µL	5.0–12.5 µL	
			Incubate cells for 1–3 days at 37°C. Then analyze transfected cells.				