# 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


invitrogen by life technologies™


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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: 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 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 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 × $10^6$ |
| | 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 |
| 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 |
| | | | | 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. | | | |