invitrogen™
by life technologies™

Lipofectamine® Reagent

Part no. 18324.lf.pps MAN0001385 **Rev. Date** 14 July 2011

Cat. nos.:	**Size:**	**Store at 4°C** (do not freeze)
18324-111	0.5 mL	
18324-012	1 mL	
18324-020	4 x 1 mL	

Description

Lipofectamine® Reagent is suitable for transfecting DNA into eukaryotic cells (1), and is a 3:1 (w/w) liposome formulation of the polycationic lipid 2,3-dioleyloxy-N-[2(sperminecarboxamido)ethyl]-N,N-dimethyl-1-propanaminium trifluoroacetate (DOSPA) and the neutral lipid dioleoyl phosphatidylethanolamine (DOPE) in membrane-filtered water. Transfection activity can be enhanced by using Plus™ Reagent (Cat. no. 11514-015) (2) to pre-complex DNA (see page 3). Refer to the Cell Lines database at **www.invitrogen.com** for protocols to transfect various cell types.

Important Guidelines for Transfection

- Prepare complexes using the amount of DNA and Lipofectamine® Reagent recommended on page 3. Optimization may be necessary. **Note:** Use Opti-MEM® I Reduced Serum Medium (Cat. no. 31985-062) to dilute Lipofectamine® Reagent and DNA before complexing.
- Transfect cells at a high cell density: We recommend 50–80% confluence at the time of transfection for high efficiency, high expression levels, and to minimize cytotoxicity. Optimization may be necessary. Maintain the same seeding conditions between experiments.
- **Do not** add antibiotics to media during transfection since this causes cell death.
- Test serum-free media for compatibility with Lipofectamine® Reagent because some serum-free formulations (e.g. CD 293, 293 SFM II, and VP-SFM) may inhibit cationic lipid-mediated transfection.

Intended Use: For research use only.
Not intended for any animal or human therapeutic or diagnostic use.

Transfection Procedure

Use the following procedure to transfect *adherent* mammalian cells in a *24-well format.* For other formats, see **Scaling Up Transfections,** on page 3. All amounts and volumes are given on a per-well basis.

1. One day before transfection, plate 2–6 × 10^4 cells in 500 µL of growth medium (with the usual amount of serum) without antibiotics so that cells will be 50–80% confluent at the time of transfection.
2. *For each transfection sample,* prepare complexes as follows:
   a. Dilute 0.2–0.4 µg DNA in 25 µL of Opti-MEM® I Reduced Serum Medium (or other medium) without serum. Mix gently.

   b. Mix Lipofectamine® Reagent gently before use, then dilute 0.5–5 µL in 25 µL of Opti-MEM® I Medium (or other medium) without serum. Mix gently.

   c. Combine the diluted DNA with diluted Lipofectamine® Reagent (total volume = 50 µL). Mix gently and incubate the plate for 15–45 minutes at room temperature (solution may appear cloudy).

   Note: Complexes are stable for 6 hours at room temperature.

   d. For each transfection, add 0.15 mL of Opti-MEM® I Medium to the tube containing the complexes (total volume = 200 µL). Mix gently.
3. Remove growth medium from cells and replace it with 0.2 mL of growth medium without serum. Add the 0.2 mL of diluted complexes (from step 2d of this procedure) to each well. Mix gently by rocking the plate.
4. Incubate the cells at 37°C in a CO_2 incubator for 2–24 hours. Start with 5 hours.
5. Add 0.4 mL of growth medium containing 2X the normal concentration of serum without removing the transfection mixture.

   Note: If toxicity is observed after transfection, replace medium with fresh, complete medium (with the normal amount of serum).
6. **For transient transfection**: Test for transgene activity 24–72 hours post-transfection as appropriate for your cell type and expression vector.

   For stable transfection: Passage cells at a 1:10 dilution into selective medium 72 hours post-transfection.

Scaling Up Transfections

To transfect cells in different tissue culture formats, vary the amounts of Lipofectamine® Reagent, DNA, cells, and medium used in proportion to the relative surface area, as shown in the following table.

Culture vessel	Relative surf. area vs. 24-well	Vol. of plating medium	DNA (μg) in media vol. (μL)	Lipofectamine® Reagent (μL) in media vol. (μL)	Transfection medium vol.
24-well	1	500 μL	0.2–0.4 μg in 25 μL	0.5–5 μL in 25 μL	0.4 mL
35-mm	5	2 mL	1–2 μg in 100 μL	2–25 μL in 100 μL	0.8 mL
60-mm	10	5 mL	3–6 μg in 300 μL	6–75 μL in 300 μL	2.4 mL
10-cm	30	15 mL	8–16 μg in 800 μL	16–200 μL in 800 μL	6.4 mL

Enhancing Transfections with Plus™ Reagent

Use this procedure to enhance transfections with Plus™ Reagent in a *24-well format.* For other formats, vary the amounts of reagents used in proportion to the relative surface area (see the preceding table).

1. Perform step 1 of the **Transfection Procedure** on page 2.
2. *For each transfection sample,* prepare complexes as follows:
   a. Dilute 0.4 μg DNA in 25 μL medium without serum (e.g. D-MEM).
   b. Mix Plus™ Reagent before use, then add 4 μL to diluted DNA. Mix again, and incubate at room temperature for 15 minutes.
   c. Dilute 1 μL Lipofectamine® Reagent in 25 μL medium without serum; mix.
   d. Combine pre-complexed DNA (from step 2b of this procedure) and diluted Lipofectamine® Reagent (from step 2c of this procedure); mix and incubate for 15 minutes at room temperature.
3. Replace the medium on cells with 0.2 mL of growth medium without serum. You may add serum at this step. Add the 54 μL of DNA-Plus™-Lipofectamine® Reagent complexes (from step 2d of this procedure) to each well of cells. Mix gently by rocking the plate back and forth.
4. Incubate cells at 37°C in a CO_2 incubator for 3 hours, and then add 0.25 mL of growth medium containing 2X the normal concentration of serum without removing the transfection mixture.
5. Continue with step 6 of the **Transfection Procedure,** page 2.

Troubleshooting

- To prevent cytotoxicity, transfect cultures at a higher confluence, use less Lipofectamine® Reagent or DNA, or include serum during transfection.
- If cells do not tolerate the absence of serum, transfect cells in the presence of serum. Prepare complexes for 45 minutes in serum-free medium, and then dilute complexes with serum-containing medium before adding to cells.

  Important: Re-optimize the amount of lipid used.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

References

1. Hawley-Nelson, P., *et al.* (1993) *Focus® 15*, 73.
2. Shih, P., *et al.* (1997) *Focus® 19*, 52.

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