

Lipofectamine™ Reagent

Cat. No. 18324-111

Cat. No. 18324-012

Cat. No. 18324-020

Size: 0.5 ml

Size: 1 ml

Size: 4 x 1 ml

Store at +4°C (do not freeze)

Description

Lipofectamine™ Reagent is suitable for the transfection of DNA into eukaryotic cells (1), and is a 3:1 (w/w) liposome formulation of the polycationic lipid 2,3-dioleoyloxy-N-[2(spermincarboxamido)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 the 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

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

Part No.: 18324.lf.pps

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This product is distributed for laboratory research only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

Transfection Procedure

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

1. One day before transfection, plate $2-6 \times 10^4$ cells in 500 μ l 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 μ g DNA in 25 μ l of Opti-MEM[®] I Reduced Serum Medium (or other medium) without serum. Mix gently.
 - b. Mix Lipofectamine[™] gently before use, then dilute 0.5-5 μ l in 25 μ l of Opti-MEM[®] I Medium (or other medium) without serum. Mix gently.
 - c. Combine the diluted DNA with diluted Lipofectamine[™] (total volume = 50 μ l). Mix gently and incubate 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 μ l). Mix gently.
3. Remove the growth medium from cells and replace with 0.2 ml of growth medium without serum. Add the 0.2 ml of diluted complexes (from Step 2d) to each well. Mix gently by rocking the plate.
4. Incubate cells at 37°C in a CO₂ incubator for 2-24 hours. We recommend starting 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 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™, DNA, cells, and medium used in proportion to the relative surface area, as shown in the table.

Culture vessel	Relative surf. area vs. 24-well	Vol. of plating medium	DNA (µg) in media vol. (µl)	Lipofectamine™ (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 table above).

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™ in 25 µl medium without serum; mix.
 - d. Combine pre-complexed DNA (Step 2b) and diluted Lipofectamine™ (Step 2c); 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 include serum at this step. Add the 54 µl of DNA-Plus™-Lipofectamine™ complexes (from Step 2d) to each well of cells. Mix gently by rocking the plate back and forth.
4. Incubate cells at 37°C in a CO₂ incubator for 3 hours, 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

1. To prevent cytotoxicity, transfect cultures at a higher confluence, use less Lipofectamine™ or DNA, or include serum during transfection.
2. If cells do not tolerate the absence of serum, transfect in the presence of serum. Prepare complexes for 45 minutes in serum-free medium, then dilute complexes with serum-containing medium before adding to cells.
Important: Re-optimize the amount of lipid used.

Quality Control

Lipofectamine™ is tested for the absence of microbial contamination using blood agar plates, Sabaraud dextrose agar plates, and fluid thioglycolate medium, and functionally by transfection of BHK-21 cells with a reporter plasmid.

References

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

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