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Lipofectin® Reagent

Part no. 18292.lipo.pps MAN0001376 **Rev. Date** 14 July 2011

Cat. nos.:	**Size:**	**Store at 4°C (do not freeze)**
18292-011	1 mL	
18292-037	4 x 1 mL	

Description

Lipofectin® Reagent is suitable for transfecting DNA (1,2), RNA (3,4), and oligonucleotides (5) into mammalian cells, and DNA into plant protoplasts (6,7). Lipofectin® Reagent is specifically recommended for transfecting endothelial cells (8). Refer to the Cell Lines database at **www.invitrogen.com** for a list of other cell types successfully transfected. Lipofectin® Reagent is a 1:1 (w/w) liposome formulation of the cationic lipid N-[1-(2,3-dioleyloxy)propyl]-n,n,n-trimethylammonium chloride (DOTMA) and dioleoyl phophotidylethanolamine (DOPE) in membrane filtered water.

Important Guidelines for Transfection

- Form complexes using the recommended amounts of DNA and Lipofectin® Reagent. Optimize as necessary (see pages 2–3).

  Note: We recommend diluting DNA and Lipofectin® Reagent into Opti-MEM® I Reduced Serum Medium (Cat. no. 31985-062) before complexing.
- Transfect cells at the recommended confluence or cell density. Optimize as necessary. Maintain the same seeding conditions between experiments.
- *Do not* add antibiotics to media during transfection; this causes cell death.
- For optimal results, transfect cells in medium without serum. Cells may be transfected in the presence of serum, if desired. However, complexes *must* be formed in serum-free medium.
- Test serum-free media for compatibility with Lipofectin® Reagent since some serum-free formulations (e.g. CD 293, 293 SFM II, 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.

Transfecting Adherent Mammalian Cells

Use the following procedure to transiently or stably transfect mammalian cells. **Note:** All amounts and volumes are given on a per-well basis.

1. One day before transfection, plate cells in growth medium without antibiotics such that they will be at the recommended confluence at the time of transfection.

Condition	Cell no.	Growth med. vol.	Format	Confluence at time of transfection
Transient	1–2 × 10^5	2 mL	6-well	40–60%
Stable	1–2 × 10^5	4 mL	60-mm	30–50%

2. **For each transfection sample**, prepare complexes as follows:
   a. Dilute 1–2 µg of DNA in 100 µL Opti-MEM® I Reduced Serum Medium (or other medium) without serum.
   b. Mix Lipofectin® Reagent before use, then dilute 2–20 µL of Lipofectin® Reagent in 100 µL Opti-MEM® I Medium (or other medium) without serum. Let stand at room temperature for 30–45 minutes.
   c. Combine the diluted DNA with diluted Lipofectin® Reagent (total volume = 200 µL). Mix gently and incubate for 10–15 minutes at room temperature (the solution may appear cloudy).
3. Remove the growth medium from the cells and wash the cells once with 2 mL of growth medium without serum. Remove the wash medium.
4. Add 0.8 mL Opti-MEM® I Medium (or other medium) without serum (1.8 mL for stable transfection) to the complexes (from step 2c of this procedure); mix gently and add to cells.
5. Incubate cells at 37°C in a CO_2 incubator for 5–24 hours.
6. Replace the medium with 2 mL of growth medium containing serum (4 mL for stable transfection). Alternatively, add 1 mL of growth medium containing 2X the normal concentration of serum (2 mL for stable transfection) without removing the DNA-containing medium.
7. **Transient:** Test for transgene expression 24–72 hours post-transfection.

   Stable cell lines: Passage cells at a 1:5 (or higher dilution) into selective medium 48–72 hours post-transfection.

Transfecting Suspension Mammalian Cells

Use the following procedure to transfect mammalian cells in suspension in a *6-well format*. All amounts and volumes are given on a per well basis.

1. On the day of transfection, prepare a single-cell suspension from stock cells. Wash the cells once with serum-free growth medium without antibiotics, and seed cells at a density of 2–3 × 10^6 cells per well in 0.8 mL of serum-free growth medium without antibiotics.
2. *For each transfection sample,* prepare complexes as follows:
   a. Dilute 1–5 µg of DNA in 100 µL of Opti-MEM® I Reduced Serum Medium (or other medium) without serum.
   b. Mix Lipofectin® Reagent before use, then dilute 2–25 µL of Lipofectin® Reagent in 100 µL of Opti-MEM® I Medium (or other medium) without serum. Let stand at room temperature for 30–45 minutes.
   c. Combine the diluted DNA with diluted Lipofectin® Reagent (total volume = 200 µL). Mix gently and incubate for 10–15 minutes at room temperature (the solution may appear cloudy).
3. Add the 200 µL of complexes to cells. Mix gently by rocking the plate back and forth.
4. Incubate cells at 37°C in a CO_2 incubator for 5–24 hours.
5. The following day, add 4 mL of complete growth medium to the cells.
6. Incubate cells at 37°C in a CO_2 incubator for 24–48 hours prior to testing for transgene expression.

Scaling Up or Down Transfections

Procedures are provided to transfect cells in a 6-well format (60-mm format for stable mammalian cell transfection). For other formats, vary the amounts of DNA, Lipofectin® Reagent, cells, and medium used in proportion to the relative surface area of the tissue culture vessel.

Optimizing Transfection

To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying cell density, DNA and Lipofectin® Reagent concentrations, and transfection incubation time.

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

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