

Lipofectamine™ 2000

Cat. No. 11668-027

Cat. No. 11668-019

Size: 0.75 ml

Size: 1.5 ml

Contents and Storage

Lipofectamine™ 2000 is supplied in liquid form at a concentration of 1 mg/ml. Store at +4°C. **DO NOT FREEZE.** Product is guaranteed for 6 months from the date of shipment if stored properly.

Description

Lipofectamine™ 2000 is a proprietary formulation suitable for the transfection of nucleic acids into eukaryotic cells. Using Lipofectamine™ 2000 for transfection provides the following advantages:

- The highest transfection efficiency in many cell types and formats (e.g. 96-well). Refer to the Transfection Collection and the Invitrogen Transfection Guide available at www.invitrogen.com for a list of cell lines and cell types that have been successfully transfected. Detailed transfection procedures are also available. For a procedure to transfect mammalian cells with short interfering RNAs (siRNA) for use in RNA interference (RNAi) studies, see www.invitrogen.com/rnai.
- DNA-Lipofectamine™ 2000 complexes can be added directly to cells in culture medium (in the presence or absence of serum).
- It is not necessary to remove complexes or change or add medium following transfection, although complexes can be removed after 4-6 hours without loss of activity.

Product Qualification

Lipofectamine™ 2000 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 CHO-K1 cells with a luciferase reporter-containing plasmid.

Important Guidelines

Follow these guidelines when performing transfections:

1. **The ratio of DNA (in µg):Lipofectamine™ 2000 (in µl) to use when preparing complexes should be 1:2 to 1:3 for most cell lines.** Some optimization may be necessary. **Example:** To transfect $0.5-2 \times 10^5$ cells in a 24-well format, use 0.8-1 µg DNA and 2-3 µl of Lipofectamine™ 2000.
2. **Transfect cells at high cell density.** 90-95% confluence at the time of transfection is recommended to obtain high efficiency and expression levels, and to minimize decreased cell growth associated with high transfection activity. Lower cell densities are suitable with optimization of conditions. Take care to maintain a standard seeding protocol between experiments because transfection efficiency is sensitive to culture confluence.
3. **Do not add antibiotics** to media during transfection as this will cause cell death.

For optimal results, we also recommend the following:

1. Use Opti-MEM® I Reduced Serum Medium (Catalog no. 31985-062) to dilute Lipofectamine™ 2000 prior to complexing with DNA. Other media without serum (e.g. D-MEM) may be used to dilute Lipofectamine™ 2000, but transfection efficiency may be compromised.
2. Test serum-free media formulations for compatibility with Lipofectamine™ 2000 as some serum-free formulations can inhibit cationic lipid-mediated transfection. CD 293, 293 SFM II, and VP-SFM are media formulations known to inhibit transfection.

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For technical questions about this product, call the Invitrogen TECH-LINESM 800 955 6288

Transfection Procedure

Use the following procedure to transfect mammalian cells in a **24-well format**. To transfect cells in other formats, see **Scaling Up or Down Transfections**.

- Adherent cells:** One day before transfection, plate $0.5\text{-}2 \times 10^5$ cells in 500 μl of growth medium without antibiotics per well so that they will be 90-95% confluent at the time of transfection.
Suspension cells: On the day of transfection just prior to preparing complexes, plate $4\text{-}8 \times 10^5$ cells in 500 μl of growth medium without antibiotics per well.
- For each transfection sample**, prepare DNA-Lipofectamine™ 2000 complexes as follows:
 - Dilute DNA in 50 μl of Opti-MEM® I Reduced Serum Medium without serum (or other medium without serum). Mix gently.
 - Mix Lipofectamine™ 2000 gently before use, then dilute the appropriate amount in 50 μl of Opti-MEM® I Medium (or other medium without serum). Mix gently and incubate for 5 minutes at room temperature. **Note:** Combine the diluted Lipofectamine™ 2000 with the diluted DNA within 30 minutes. Longer incubation times may decrease activity. If D-MEM is used as a diluent for the Lipofectamine™ 2000, mix with the diluted DNA within 5 minutes.
 - After the 5 minute incubation, combine the diluted DNA with the diluted Lipofectamine™ 2000 (total volume is 100 μl). Mix gently and incubate for 20 minutes at room temperature to allow the DNA-Lipofectamine™ 2000 complexes to form. The solution may appear cloudy, but this will not inhibit the transfection. **Note:** DNA-Lipofectamine™ 2000 complexes are stable for 6 hours at room temperature.
- Add the 100 μl of DNA-Lipofectamine™ 2000 complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.
- Incubate the cells at 37°C in a CO₂ incubator for 24-48 hours until they are ready to assay for transgene expression. It is not necessary to remove the complexes or change the medium; however, growth medium may be replaced after 4-6 hours without loss of transfection activity.
- For stable cell lines:** Passage the cells at a 1:10 or higher dilution into fresh growth medium 24 hours after transfection. Add selective medium the following day.
For suspension cells: Add PMA and/or PHA (if desired) 4 hours after adding the DNA-Lipofectamine™ 2000 complexes to the cells. **Tip:** For Jurkat cells, adding PHA-L and PMA at final concentrations of 1 $\mu\text{g}/\text{ml}$ and 50 ng/ml, respectively, enhances CMV promoter activity and gene expression. For K562 cells, adding PMA alone is sufficient to enhance promoter activity.

Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of Lipofectamine™ 2000, DNA, cells, and medium used in proportion to the difference in surface area (see table). With automated, high-throughput systems, larger complexing volumes are recommended for transfections in 96-well plates. **Note:** You may perform rapid 96-well plate transfections (plate cells and transfect simultaneously) by adding a suspension of cells directly to complexes prepared in the plate. Prepare complexes and add cells at twice the cell density as in the basic protocol in a 100 μl volume. Cells will adhere as usual in the presence of DNA-Lipofectamine™ 2000 complexes.

| Culture Vessel | Surface Area per Well (cm ²) | Relative Surface Area (vs. 24-well) | Volume of Plating Medium | DNA (μg) and Dilution Volume (μl) | Lipofectamine™ 2000 (μl) and Dilution Volume (μl) |
|----------------|--|-------------------------------------|--------------------------|---|---|
| 96-well | 0.3 | 0.2 | 100 μl | 0.2 μg in 25 μl | 0.5 μl in 25 μl |
| 24-well | 2 | 1 | 500 μl | 0.8 μg in 50 μl | 2.0 μl in 50 μl |
| 12-well | 4 | 2 | 1 ml | 1.6 μg in 100 μl | 4.0 μl in 100 μl |
| 35-mm | 10 | 5 | 2 ml | 4.0 μg in 250 μl | 10 μl in 250 μl |
| 6-well | 10 | 5 | 2 ml | 4.0 μg in 250 μl | 10 μl in 250 μl |
| 60-mm | 20 | 10 | 5 ml | 8.0 μg in 0.5 ml | 20 μl in 0.5 ml |
| 10-cm | 60 | 30 | 15 ml | 24 μg in 1.5 ml | 60 μl in 1.5 ml |

Note: Surface areas are determined from actual measurements of tissue culture vessels.

Optimizing Transfection

To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying DNA and Lipofectamine™ 2000 concentrations, and cell density. Make sure that cells are greater than 90% confluent and vary DNA (μg):Lipofectamine™ 2000 (μl) ratios from 1:0.5 to 1:5.