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# Lipofectamine® 2000 CD Reagent

**Part no.** 12566014.pps MAN0001090 **Rev. Date** 14 July 2011

**Cat. no.** 12566-014 **Size** 1.0 mL **Store at 4°C (do not freeze)**

## Description

Lipofectamine® 2000 CD is a proprietary *animal origin-free* formulation for transfecting nucleic acids into eukaryotic cells. A Drug Master File (DMF) has been submitted to the FDA. Contact Life Technologies Technical Service or your local Sales Representative for permission to cross-reference the DMF. Using Lipofectamine® 2000 CD for transfection provides the following advantages:

- Highest transfection efficiency in many cell types and formats. Refer to the Cell Lines database at www.invitrogen.com for a list of cell types transfected.
- The animal origin-free formulation ensures that mammalian cell culture and bioproduction processes are free of animal-derived materials.
- DNA-Lipofectamine® 2000 CD complexes can be added directly to cells in culture medium.
- It is not necessary to remove complexes or change/add medium after transfection, but complexes may be removed after 4–6 hours.

## Important Guidelines for Transfection

- Culture cells in serum-free media that are free of animal-derived components. Test serum-free media for compatibility with Lipofectamine® 2000 CD since some serum-free formulations (e.g. CD 293, 293 SFM II, CD Hybridoma) may inhibit cationic lipid-mediated transfection.
- For consistent animal origin-free transfection, use OptiPro™ SFM (Cat. no. 12309-019) to dilute DNA and Lipofectamine® 2000 CD before complexing.
- Transfect cells at high cell density: For adherent cells, we recommend 90–95% confluence at the time of transfection for high efficiency, high expression levels, and to minimize cytotoxicity. For suspension cultures, transfect cells at a density of 0.8–1.1 × $10^6$ cells/mL. Optimization may be necessary. Maintain the same seeding conditions between experiments.
- *Do not* add antibiotics to media during transfection since this causes cell death.
- *Do not* add Pluronic® or charged media extracts (e.g. dextran sulfate) to media during transfection, as these reagents can inhibit transfection.

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

## Transfecting Adherent Cells

Use the following procedure to transfect mammalian cells in a *24-well format*. For other formats, see **Scaling Up or Down Transfections** (page 3).Use the recommended Lipofectamine® 2000 CD amount as a starting point, and then optimize conditions for your cell line and serum-free medium, as needed. All amounts and volumes are given on a per-well basis.

1. One day before transfection, plate 0.5–2 × $10^5$ cells in 500 µL of growth medium without antibiotics so that they will be 90–95% confluent at the time of transfection.
2. ***For each transfection sample***, prepare complexes as follows:
   a. Dilute DNA in 50 µL of OptiPro™ SFM. Mix gently.
   b. Mix Lipofectamine® 2000 CD gently before use, then dilute the appropriate amount in 50 µL of OptiPro™ SFM. Incubate for 5 minutes at room temperature.

      **Note:** Combine diluted Lipofectamine® 2000 CD with diluted DNA within 30 minutes.
   c. After 5 minute incubation, combine the diluted DNA with diluted Lipofectamine® 2000 CD (total volume = 100 µL). Mix gently and incubate for 20 minutes at room temperature (solution may appear cloudy).

      **Note:** Complexes are stable for 6 hours at room temperature.
3. Add the 100 µL of complexes to a well containing cells and medium. Mix gently by rocking the plate back and forth.
4. Incubate cells at 37°C in a $CO_2$ incubator for 18–48 hours prior to testing for transgene expression. It is not necessary to change the medium, but medium may be replaced after 4–6 hours.
5. *For stable cell lines:* Passage cells at a 1:10 (or higher dilution) into fresh growth medium 24 hours after transfection. Add selective medium the following day.

## Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of Lipofectamine® 2000 CD, DNA, cells, and medium used in proportion to the relative surface area, as shown in the following table. With automated, high-throughput systems, we recommend a complexing volume of 50 µL for transfections in 96-well plates.

| Culture vessel | Surf. area per well ($cm^2$) | Relative surf. area vs. 24-well | Vol. of plating medium | DNA (µg) in media vol. (µL) | Lipofectamine® 2000 CD (µL) in media vol. (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, and may vary depending on the manufacturer.

## Transfecting Cells in Suspension

Use the following procedure to transfect suspension cells at any scale. Before beginning, make sure that cells are healthy and > 90% viable.

1. On the day of transfection, prepare a single-cell suspension from stock cells at less than $3 \times 10^6$ cells/mL. Seed cells at a density of $0.8–1.1 \times 10^6$ cells/mL in growth media without antibiotics.
2. *For each transfection sample,* prepare complexes as described in step 2 of **Transfecting Adherent Cells** on page 2, using the following reagent amounts and volumes *for every mL of cells transfected:*
   - Dilute 0.5–1.5 µg DNA in 34 µL of OptiPro™ SFM
   - Dilute 1–10 µL of Lipofectamine® 2000 CD in 34 µL of OptiPro™ SFM
3. Add the complexes to the flask containing cells and media.
4. Incubate the cells at 37°C in a $CO_2$ incubator on an orbital shaker rotating at 125 rpm for 24–96 hours prior to testing for transgene expression.

## Optimizing Transfection

To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying cell density and Lipofectamine® 2000 CD concentrations.

## 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.

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