

Lipofectamine[®] 2000 Transfection Reagent

Cat. nos:	Size:	Store at 4°C (do not freeze)
11668-027	0.75 mL	
11668-019	1.5 mL	
11668-500	15 mL	
Part no. 11668.2k.pps	MAN0000995	Rev. Date 14 July 2011

Description

Lipofectamine[®] 2000 Transfection Reagent is a proprietary formulation for transfecting nucleic acids (DNA and RNA) into eukaryotic cells and provides the following advantages:

- Highest transfection efficiency in many cell types and formats (e.g. 96-well). Refer to the Cell Lines database at www.invitrogen.com for a list of cell types successfully transfected.
- Nucleic acid-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/add medium after transfection, but complexes may be removed after 4–6 hours.

Important Guidelines for Transfection

- Use the procedure in **Transfect siRNA, Including Stealth RNAi[®] siRNA** on page 2 to transfect cells with short interfering RNA (siRNA).
- Use the procedure in **Transfect Plasmid DNA** on page 3 to transfect cells with plasmid DNA.
- We recommend Opti-MEM[®] I Reduced Serum Medium (Cat. no. 31985-062) to dilute Lipofectamine[®] 2000 Transfection Reagent and nucleic acids.
- *Do not* add antibiotics to media during transfection.
- Maintain the same seeding conditions between experiments.
- Test serum-free media for compatibility with Lipofectamine[®] 2000 Transfection Reagent since some serum-free formulations (e.g. CD293, SFM II, VP-SFM) may inhibit cationic lipid-mediated transfection.

Note: For more tips for your RNAi experiment, refer to *Seven Steps to RNAi Success*. This manual is available from www.invitrogen.com/rnai or Technical Service, as are cell-type specific RNAi transfection protocols (see *RNAi protocols*.)

Intended Use: For research use only.

Not intended for any animal or human therapeutic or diagnostic use.

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.

Transfect siRNA, Including Stealth RNAi® siRNA

Use this procedure to transfect siRNA, such as **Stealth RNAi® siRNA**, into mammalian cells in a *24-well format*. For other formats, see **Scaling Up or Down Transfections** on page 4. All amounts and volumes are given on a per-well basis. Optimize transfections as described in **Optimizing siRNA and Stealth RNAi® siRNA Transfection**.

1. One day before transfection, plate cells in 500 μL of growth medium without antibiotics such that they will be *30–50% confluent* at the time of transfection.
Note: Transfecting cells at a lower density allows a longer interval between transfection and assay time, and minimizes the loss of cell viability due to cell overgrowth.
2. **For each transfection sample**, prepare oligomer-Lipofectamine® 2000 Transfection Reagent complexes as follows:
 - a. Dilute 20 pmol siRNA oligomer, such as Stealth RNAi® siRNA, in 50 μL Opti-MEM® I Reduced Serum Medium without serum (final concentration of RNA when added to the cells is 33 nM). Mix gently.
 - b. Mix Lipofectamine® 2000 Transfection Reagent gently before use, then dilute 1 μL in 50 μL Opti-MEM® I Reduced Serum Medium. Mix gently and incubate for 5 minutes at room temperature.
Note: Proceed to step 2c within 25 minutes.
 - c. After the 5-minute incubation, combine the diluted oligomer with the diluted Lipofectamine® 2000 Transfection Reagent. Mix gently and incubate for 20 minutes at room temperature (the solution may appear cloudy).
3. Add the oligomer-Lipofectamine® 2000 complexes to each well containing cells and medium. Mix by gently rocking the plate.
4. Incubate the cells at 37°C in a CO₂ incubator for 24–96 hours until you are ready to assay for gene knockdown. The medium may be changed after 4–6 hours.

Optimizing siRNA and Stealth RNAi® siRNA Transfection

To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying RNA and Lipofectamine® 2000 Transfection Reagent concentrations. Test 10–50 pmol RNA and 0.5–1.5 μL Lipofectamine® 2000 Transfection Reagent for 24-well format. Also consider transfecting cells at higher densities when optimizing conditions.

Transfect Plasmid DNA

Use the following procedure to transfect DNA into mammalian cells in a *24-well format*. For other formats, see **Scaling Up or Down Transfections** on page 4. All amounts and volumes are given on a per-well basis. Prepare complexes using a DNA (μg) to Lipofectamine[®] 2000 Transfection Reagent (μL) ratio of 1:2 to 1:3 for most cell lines. Transfect cells at high cell density for high efficiency, high expression levels, and to minimize cytotoxicity. Optimization may be necessary (see **Optimizing Plasmid DNA Transfection** on page 4).

1. **Adherent cells:** One day before transfection, plate $0.5\text{--}2 \times 10^5$ cells in 500 μL of growth medium without antibiotics so that cells will be 90–95% confluent at the time of transfection.

Suspension cells: Just prior to preparing complexes, plate $4\text{--}8 \times 10^5$ cells in 500 μL of growth medium without antibiotics.

2. *For each transfection sample*, prepare complexes as follows:

- a. Dilute DNA in 50 μL of Opti-MEM[®] I Reduced Serum Medium without serum (or other medium without serum). Mix gently.
- b. Mix Lipofectamine[®] 2000 Transfection Reagent gently before use, then dilute the appropriate amount in 50 μL of Opti-MEM[®] I Reduced Serum Medium without serum. Incubate for 5 minutes at room temperature.

Note: Proceed to step 2c within 25 minutes.

- c. After the 5 minute incubation, combine the diluted DNA with diluted Lipofectamine[®] 2000 Transfection Reagent (total volume = 100 μL). Mix gently and incubate for 20 minutes at room temperature (the solution may appear cloudy).

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

3. Add the 100 μL of complexes to each well containing cells and medium. Mix gently by rocking the plate back and forth.

Note: Although not required, you may replace the old growth mediums added the day before transfection (step 1 of this procedure) with fresh medium without antibiotics. If you are not using serum during transfection, replace the old medium with serum-free medium.

4. Incubate cells at 37°C in a CO₂ incubator for 18–48 hours prior to testing for transgene expression. The medium may be changed 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 (if desired) the following day.

Optimizing Plasmid DNA Transfection

To obtain the highest transfection efficiency and low cytotoxicity, optimize transfection conditions by varying DNA (μg): Lipofectamine[®] 2000 Transfection Reagent (μL) ratios from 1:0.5 to 1:5. Use cells that are more than 90% confluent.

Scaling Up or Down Transfections

To transfect cells in different tissue culture formats, vary the amounts of Lipofectamine[®] 2000 Transfection Reagent, nucleic acid, cells, and medium used in proportion to the relative surface area (see the following table). With automated, high-throughput systems, a complexing volume of 50 μL is recommended for transfections in 96-well plates. **Note:** You may perform rapid 96-well plate transfections by plating cells directly into the transfection mix. Prepare complexes in the plate and directly 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 complexes.

Culture vessel	Surf. area per well ¹	Shared reagents		DNA transfection		RNAi transfection	
		Vol. of plating medium	Vol. of dilution medium ²	DNA	Lipofectamine [®] 2000	RNA	Lipofectamine [®] 2000
96-well	0.3 cm ²	100 μL	2 \times 25 μL	0.2 μg	0.5 μL	5 pmol	0.25 μL
24-well	2 cm ²	500 μL	2 \times 50 μL	0.8 μg	2.0 μL	20 pmol	1.0 μL
12-well	4 cm ²	1 mL	2 \times 100 μL	1.6 μg	4.0 μL	40 pmol	2.0 μL
6-well	10 cm ²	2 mL	2 \times 250 μL	4.0 μg	10 μL	100 pmol	5 μL
60-mm	20 cm ²	5 mL	2 \times 0.5 mL	8.0 μg	20 μL	200 pmol	10 μL
10-cm	60 cm ²	15 mL	2 \times 1.5 mL	24 μg	60 μL	600 pmol	30 μL

¹ Surface areas may vary depending on the manufacturer.

² Volumes of dilution medium in step 2a & 2b of DNA or RNAi transfection protocols.

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