

Transfection HepG2 Version 2

IGEM AFCM-EGYPT

Abstract

Lipofectamine™ 2000 Transfection Protocol

Description

Lipofectamine™ 2000 is a proprietary formulation for the transfection of nucleic

acids (DNA and RNA) into eukaryotic cells providing 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.

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Protocol

Step 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.

Step 2.

For each transfection sample, prepare oligomer-Lipofectamine™ 2000 complexes as follows: a. Dilute 20 pmol Stealth™ RNAi or siRNA oligomer in 50 µl Opti-MEM® Reduced Serum Medium without serum (resulting concentration of RNAi is 40 nM). Mix gently. b. Mix Lipofectamine™ 2000 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 c within 25 minutes. c. After the 5-minute incubation, combine the diluted oligomer with the diluted Lipofectamine™ 2000. Mix gently and incubate for 20 minutes at room temperature (solution may appear cloudy).

Step 3.

Add the oligomer-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 24-96 hours until you are ready to assay for gene knockdown. Medium may be changed after 4-6 hours. Optimizing Stealth™ RNAi or siRNA Transfection To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying RNA and Lipofectamine™ 2000 concentrations. Test 10-50 pmol RNA and 0.5-1.5 µl Lipofectamine™ 2000 for 24-well format. Depending on the nature of the target gene, transfecting cells at higher densities may also be considered when optimizing conditions. Page 3 Plasmid DNA Transfection Use the following procedure to transfect DNA into mammalian cells in a 24-well format. For other formats, see Scaling Up or Down Transfections (page 4). All amounts and volumes are given on a per well basis. Prepare complexes using a DNA (µg) to Lipofectamine™ 2000 (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, page 4).

Step 4.

Adherent cells: One day before transfection, plate 0.5-2 x 10⁵ 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 4-8 x 10⁵ cells in

Step 5.

µl of growth medium without antibiotics.

Step 6.

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 gently before use, then dilute the appropriate amount in 50 µl of Opti-MEM® I Medium. Incubate for 5 minutes at room temperature. Note: Proceed to Step c within 25 minutes. c. After the 5 minute incubation, combine the diluted DNA with diluted Lipofectamine™ 2000 (total volume = 100 µl). Mix gently and incubate for

Step 7.

minutes at room temperature (solution may appear cloudy). Note: Complexes are stable for 6 hours at room temperature.

Step 8.

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

Step 9.

Incubate cells at 37°C in a CO2 incubator for 18-48 hours prior to testing for transgene expression. Medium may be changed after 4-6 hours.

Step 10.

Surface areas may vary depending on the manufacturer.

Step 11.

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. Page 4 Optimizing Plasmid DNA Transfection To obtain the highest transfection efficiency and low cytotoxicity, optimize transfection conditions by varying cell density as well as DNA and Lipofectamine™ 2000 concentrations. Make sure

that cells are greater than 90% confluent and vary DNA (µg): Lipofectamine™ 2000 (µl) ratios from 1:0.5 to 1:5. Scaling Up or Down Transfections To transfect cells in different tissue culture formats, vary the amounts of Lipofectamine™ 2000, nucleic acid, cells, and medium used in proportion to the relative surface area, as shown in the 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 Vol. of plating medium	2 Vol. of dilution medium	DNA	Lipofectamine™ 2000	RNA
96-well	0.3 cm ²	100 µl	2 x 25 µl	0.2 µg	0.5 µl	5 pmol	0.25 µl
24-well	2 cm ²	500 µl	2 x 50 µl	0.8 µg	2.0 µl	20 pmol	1.0 µl
12-well	4 cm ²	1 ml	2 x 100 µl	1.6 µg	4.0 µl	40 pmol	2.0 µl
6-well	10 cm ²	2 ml	2 x 250 µl	4.0 µg	10 µl	100 pmol	5 µl
60-mm	20 cm ²	5 ml	2 x 0.5 ml	8.0 µg	20 µl	200 pmol	10 µl
10-cm	60 cm ²	15 ml	2 x 1.5 ml	24 µg	60 µl	600 pmol	30 µl

Step 12.

Volumes of dilution medium in Step 2a & 2b of DNA or RNAi transfection protocols. Purchaser Notification This product is covered by one or more Limited Use Label Licenses (see the Invitrogen catalog or our web-site, www.invitrogen.com). By the use of this product you accept the terms and conditions of all applicable Limited Use Label Licenses. Limited Use Label License No. 27: Lipofectamine™ 2000 Reagent Limited Use Label License No. 173: Inhibition of Gene Expression by Double-Stranded RNA Limited Use Label License No. 196: Stealth™ RNAi ©2000-2005 Invitrogen Corporation. All rights reserved.