untitled protocol

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 withoutantibiotics such that they will be 30-50% confluent at the time of transfection.Note: Transfecting cells at a lower density allows a longer interval betweentransfection and assay time, and minimizes the loss of cell viability due to cellovergrowth.

Step 2.

For each transfection sample, prepare oligomer-Lipofectamine™ 2000complexes as follows:a. Dilute 20 pmol Stealth™ RNAi or siRNA oligomer in 50 µl Opti-MEM® IReduced Serum Medium without serum (resulting concentration of RNAis 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 minutesat room temperature. Note: Proceed to Step c within 25 minutes.c. After the 5-minute

incubation, combine the diluted oligomer with thediluted Lipofectamine™ 2000. Mix gently and incubate for 20 minutes atroom temperature (solution may appear cloudy).

Step 3.
Add the oligomer-Lipofectamine™ 2000 complexes to each well containingcells 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 readyto assay for gene knockdown. Medium may be changed after 4-6 hours.Optimizing Stealth™ RNAi or siRNA TransfectionTo obtain the highest transfection efficiency and low non-specific effects,optimize transfection conditions by varying RNA and Lipofectamine™ 2000concentrations. 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 athigher densities may also be considered when optimizing conditions.Page 3Plasmid DNA TransfectionUse the following procedure to transfect DNA into mammalian cells in a 24-wellformat. For other formats, see Scaling Up or Down Transfections (page 4). Allamounts and volumes are given on a per well basis. Prepare complexes using aDNA (µg) to Lipofectamine™ 2000 (µl) ratio of 1:2 to 1:3 for most cell lines. Transfectcells at high cell density for high efficiency, high expression levels, and to minimizecytotoxicity. Optimization may be necessary (see Optimizing Plasmid DNATransfection, page 4).

Step 4.
Adherent cells: One day before transfection, plate 0.5-2 x 105 cells in 500 µl ofgrowth medium without antibiotics so that cells will be 90-95% confluent atthe 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 withoutserum (or other medium without serum). Mix gently.b. Mix Lipofectamine™ 2000 gently before use, then dilute the appropriateamount in 50 µl of Opti-MEM® I Medium. Incubate for 5 minutes at roomtemperature. Note: Proceed to Step c within 25 minutes.c. After the 5 minute incubation, combine the diluted DNA with dilutedLipofectamine™ 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. Mixgently 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 fortransgene 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 freshgrowth medium 24 hours after transfection. Add selective medium (ifdesired) the following day.Page 4Optimizing Plasmid DNA TransfectionTo obtain the highest transfection efficiency and low cytotoxicity, optimizetransfection conditions by varying cell density as well as DNA andLipofectamine™ 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 TransfectionsTo transfect cells in different tissue culture formats, vary

the amounts ofLipofectamine™ 2000, nucleic acid, cells, and medium used in proportion to therelative surface area, as shown in the table. With automated, high-throughputsystems, a complexing volume of 50 µl is recommended for transfections in 96-well plates. Note: You may perform rapid 96-well plate transfections by platingcells directly into the transfection mix. Prepare complexes in the plate anddirectly add cells at twice the cell density as in the basic protocol in a 100 µlvolume. Cells will adhere as usual in the presence of complexes.Culture Shared reagents DNA transfection RNAi transfectionvesselSurf.area perwell1Vol. ofplatingmediumVol. ofdilutionmedium2DNA Lipofectamine™2000RNA Lipofectamine™200096-well 0.3 cm2 100 µl 2 x 25 µl 0.2 µg 0.5 µl 5 pmol 0.25 µl24-well 2 cm2 500 µl 2 x 50 µl 0.8 µg 2.0 µl 20 pmol 1.0 µl12-well 4 cm2 1 ml 2 x 100 µl 1.6 µg 4.0 µl 40 pmol 2.0 µl6-well 10 cm2 2 ml 2 x 250 µl 4.0 µg 10 µl 100 pmol 5 µl60-mm 20 cm2 5 ml 2 x 0.5 ml 8.0 µg 20 µl 200 pmol 10 µl10-cm 60 cm2 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 NotificationThis product is covered by one or more Limited Use Label Licenses (see the Invitrogencatalog or our web-site, www.invitrogen.com). By the use of this product you acceptthe terms and conditions of all applicable Limited Use Label Licenses.Limited Use Label License No. 27: Lipofectamine™ 2000 ReagentLimited Use Label License No. 173: Inhibition of Gene Expression by Double-Stranded RNALimited Use Label License No. 196: Stealth™ RNAi