Lip2000 Transfection Reagent

Cat. AC12023

Description

Lip2000 is a newly developed and proprietary reagent for the transfection of nucleic acids into eukaryotic cells.

Lip2000 has the following advantages:

The highest transfection efficiency in many cell types and formats.

DNA-Lip2000 complexes can be directly added to cells in culture medium (with or without serum).

It is not necessary to remove DNA-Lip2000complexes or change medium following transfection.

The complexes can be removed after 4-6 hours by replacing with refresh medium (optional)

Contents and Storage

Lip2000 is supplied in liquid form at a concentration of 1mg/ml. Store at 4℃. **DO NOT FREEZE**.

Product Qualification

Lip2000 has been extensively tested by transfection of HEK293 cells with an EGFP reporter containing plasmid. Lip2000is free of microbial contamination.

Important Guidelines

Follow these guidelines when performing transfections:

1. The ratio of DNA (in µg) : Lip2000 (in µl) to use when preparing complexes should be 1:2 to 1:3 for most cell lines. 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 Lip2000. Optimizing transfection by varying DNA/Lip2000 ratio is possible.
2. It is **CRITICAL** to transfect cells at high cell density. 90-95% confluence 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 dependent on culture confluence.
3. **DO NOT** add antibiotics to media during transfection as this will cause cell death.

For better results, you may choose to:

Use Opti-MEM I medium to dilute Lip2000 prior to complexing with DNA. Other media without serum (e.g.DMEM) may be used to dilute Lip2000,but transfection efficiency may be compromised.

Note: Some serum-free formulations can inhibit Lip2000mediated transfection, for example:CD 293, 293 SFM II, and VP-SFM etc.

Transfection Procedure for 24-Well Format

For adherent cells: One day before transfection,plate cells in growth medium (without antibiotics) so that they will be 90-95% confluent at the time of transfection (0.5 -2 $\times 10^5$ cells/well for a 24-well plate).

For suspension cells: On the day of transfection just prior to preparing complexes,plate 4-8$\times 10^5$cells/500 µl of growth medium (without antibiotics) in a 24-well plate.

1. For each transfection sample, prepare DNA-Lip2000complexes as follows:

• Dilute DNA in 50 µl of Opti-MEM I Reduced Serum Medium without serum (or other medium without serum). Mix gently.

• Mix Lip2000gently 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 Lip2000 with the diluted DNA within 30 minutes. Longer incubation times may decrease activity. If DMEM is used as a diluent for the Lip2000, mix with the diluted DNA within 5 minutes. After the 5 minute incubation,combine the diluted DNA with the diluted Lip2000(total volume is 100 µl).
•Mix gently and incubate for 20 minutes at room temperature to allow the DNALip2000 complexes to form. The solution may appear cloudy,but this will not inhibit the transfection.
Note:DNA-Lip2000 complexes are stable for at least 5 hours at room temperature.
2. Add the 100 µl of DNA-Lip2000 complexes to each well. Mix gently by rocking the plate back and forth.
3. Incubate the cells at 37℃ in a CO_2 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-Lip2000 complexes to the cells.
Tip: For Jurkat cells, adding PHA-L and PMA at final concentrations of 1 µg/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 Lip2000, DNA, cells, and medium used in proportion to the difference in surface area (see table below). With automated, highthroughput 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

Culture Vessel	Surface Area per Well (cm^2)	Relative Surface Area (vs. 24-well)	Volume of Plating Medium	DNA (µg) and Dilution Volume (µl)	Lip2000(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 in250 µl	10 µl in 250 µl
6-well	10	5	2 ml	4.0 µg in250 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 Lip2000 concentrations, and cell number. Make sure that cells are greater than 90% confluent and vary DNA (µg) : Lip2000(µl) ratios from 1:0.5 to 1:5.