LipoTAXI® Mammalian Transfection Kit

INSTRUCTION MANUAL

Catalog #204110

Revision B.0

For Research Use Only. Not for use in diagnostic procedures.

204110-12

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, express or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Agilent. Agilent shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

ORDERING INFORMATION AND TECHNICAL SERVICES

United States and Canada

Agilent Technologies
Stratagene Products Division
11011 North Torrey Pines Road
La Jolla, CA 92037
Telephone (858) 373-6300
Order Toll Free (800) 424-5444
Technical Services (800) 894-1304
Internet techservices@agilent.com
World Wide Web www.stratagene.com

Europe

Location	Telephone	Fax	Technical Services
Austria	0800 292 499	0800 292 496	0800 292 498
Belgium	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 15775	0800 15740	0800 15720
France	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 919 288	0800 919 287	0800 919 289
Germany	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 182 8232	0800 182 8231	0800 182 8234
Netherlands	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 023 0446	+31 (0)20 312 5700	0800 023 0448
Switzerland	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 563 080	0800 563 082	0800 563 081
United Kingdom	00800 7000 7000	00800 7001 7001	00800 7400 7400
	0800 917 3282	0800 917 3283	0800 917 3281

All Other Countries

Please contact your local distributor. A complete list of distributors is available at www.stratagene.com.

LipoTAXI® Mammalian Transfection Kit

CONTENTS

LipoTAXI® Mammalian Transfection Kit

MATERIALS PROVIDED

Materials provided	Composition	Quantity
LipoTAXI® transfection reagent	Lipid solution (3 μmol)	3 × 1-ml tubes
Control plasmid	pCMV β-gal	30 μg (1 μg/μl)

STORAGE CONDITIONS

LipoTAXI® Transfection Reagent: Room temperature

Control Plasmid: –20°C

Note *Do not freeze the LipoTAXI® transfection reagent.*

ADDITIONAL MATERIALS REQUIRED

Dulbecco's Modified Eagle Medium (DMEM)
Phosphate-buffered saline (PBS)[§]
o-Nitrophenyl-β-D-galactopyranoside (ONGP) substrate solution[§]

[§]See *Preparation of Media and Reagents.*

Revision B.0

INTRODUCTION

Lipid transfection techniques have found many applications in gene transfer experiments with mammalian and invertebrate cells. The art of lipid transfection is to strike a balance between optimal transfection efficiency and cytotoxicity of the lipid–DNA complex. The LipoTAXI® mammalian transfection kit contains a unique, low-toxicity lipid formulation that has been tested with over 30 cell lines (see Table I) and may be used with both adherent cells and cells growing in suspension. The kit has been optimized for 30–85 transfections in 60-mm tissue culture dishes.

TABLE I

Cell Lines Tested with LipoTAXI® Transfection Reagent

Cell line	Tissue type/species	Relative transfectability[a]
3T3-NIH	Fibroblast, mouse	++
BHK	Kidney, hamster	+++
CHO	Ovary, hamster	+++
COS-1	Kidney SV-40 transformed, African green monkey	++++
DU 145	Prostate carcinoma, metastasis to brain, human	++
DUPRO	Prostate cancer, human	+++
NCI-H441	Lung cancer, human	++
HeLa	Cervical carcinoma, human	++
JAR	Choriocarcinoma, human	++
Jurkat	Acute T cell leukemia, human	++
LNCaP	Prostate cancer, human	++
MDA-MB-453	Breast carcinoma, human	++
PC-3	Prostate cancer, human	+
PPC-1	Prostate cancer, human	+++
Saos-2	Osteosarcoma, human	++
SK-N-BE(2)	Neuroblastoma, human	++
T-47D	Breast ductal carcinoma, human	++

[a] A single plus (+) indicates the β-galactosidase activity in cell extract transiently transfected with CMV β-gal was at least one order of magnitude higher than activity from cells transfected without DNA.

TRANSFECTION PROTOCOL

The following procedure for the generation of transient transfections uses 60-mm tissue culture dishes. See Table II for reagent volumes for dishes of other dimensions. The optimal ratio of LipoTAXI transfection reagent to DNA must be determined for each plasmid and cell line.

Transfecting Adherent Cells with the LipoTAXI Mammalian Transfection Kit

Preparing the Cells for Transfection

1. Twenty-four hours before transfection, inoculate a 60-mm tissue culture dish with 4×10^5–11×10^5 exponentially growing cells. The cells should be 60–80% confluent at the time of transfection.

2. Grow the cells overnight in 5 ml of the appropriate culture medium.

Preparing the Complex Formation Solution

1. Transfer 900 µl of sterile serum-free, antibiotic-free DMEM (DMEM–SA) to a polystyrene tube.

   Note *Polystyrene tubes yield superior results to polyethylene or polypropylene tubes.*

2. Add 35–100 µl of LipoTAXI transfection reagent. Tap the side of the tube to mix.

3. Add 7 µg of the control plasmid to the control reaction and 5–10 µg of the experimental DNA to the experimental reaction. For stable transfections, prepare a negative control.

4. Mix gently (do not vortex) and incubate for 15–30 minutes at room temperature.

TABLE II

Tissue culture dish format	Diameter of the well (mm)	Recommended number of adherent cells ($\times 10^5$)	Volume of LipoTAXI transfection reagent (µl/well)	Final volume of complex formation solution (µl/well)	Final volume of activated solution (µl/well)
96-well	6.4	0.04–0.1	0.4–1.2	4	10
48-well	10	0.1–0.3	1.0–3.0	8	20
24-well	15	0.27–0.67	2.5–7.5	30	80
12-well	22	0.6–1.5	5–15	100	300
6-well	35	1.5–3.7	12–35	300	750
60-mm	60	4.0–11.0	35–100	1000	2500
100-mm	100	12.0–31.0	100–300	3000	8000
150-mm	150	27.0–69.0	200–600	6000	16000

Adding the Activated Solution

1. Remove the standard culture medium from the tissue culture dish by aspiration.

2. Add 1.5 ml of DMEM (serum optional) to the transfection mixture in the polystyrene tube and then transfer this entire mixture (~2.5 ml) dropwise to the tissue culture dish while swirling the dish.

3. Incubate for 4–6 hours using standard growth conditions (i.e., 37°C and 5% CO_2 in a humidified incubator).

4. Add 2.5 ml of DMEM containing serum (DMEM+S) at twice the normal serum concentration to the tissue culture dish and incubate overnight.

5. Replace the medium with 5.0 ml of fresh, complete medium.

6. Incubate the cells for 24–72 hours depending on the cell type, reporter system, and promoter activity or proceed to *Performing a Stable Transfection*.

Performing a Stable Transfection

1. Split the cells from step 6 of *Adding the Activated Solution* to the desired ratio (at least 1:10) after the 24-hour incubation and then incubate overnight.

2. Apply selection antibiotics dropwise to the tissue culture dish, swirling the dish between drops, at a concentration appropriate to the cell line.

3. Replace the medium and apply fresh selection antibiotics every 4–7 days (approximately two times per week).

4. Stable colonies form within 1–2 weeks. Cells from the negative DNA control dish die off.

Transfecting Suspension Cells with the LipoTAXI Mammalian Transfection Kit

Preparing the Cells for Transfection

1. Seed 4×10^6–10×10^6 cells per 60-mm dish in 700 µl of DMEM–SA.

   Note *The optimal ratio of LipoTAXI transfection reagent to DNA must be determined for each plasmid and cell line.*

Preparing the Complex Formation Solution

1. Transfer 900 µl of DMEM–SA to a polystyrene tube.

2. Add 35–100 µl of LipoTAXI transfection reagent. Tap the side of the tube to mix.

3. Add 10 µg of the control plasmid to the control reaction and 7–15 µg of the experimental DNA to the experimental reaction. For stable transfections, prepare a negative control.

4. Mix gently (do not vortex) and incubate for 15–30 minutes at room temperature.

Adding the Activated Solution

1. Add 800 µl of DMEM (serum optional) to the transfection mixture in the polystyrene tube and then transfer this entire mixture (~1.8 ml) dropwise to the tissue culture dish while swirling the dish.

2. Incubate for 4–6 hours using standard growth conditions (i.e., 37°C and 5% CO_2 in a humidified incubator).

3. Add 3 ml of fresh, complete DMEM to the tissue culture dish.

4. Incubate the cells for 24–72 hours depending on the cell type, reporter system, and promoter activity or proceed to step 10 if performing a stable transfection.

Performing a Stable Transfection

1. After 48 hours, seed a fresh tissue culture dish to be used for selection at no greater than one-third the density of the transfected cells from step 9.

2. Apply selection antibiotics dropwise to the tissue culture dish, swirling the dish between drops, at a concentration appropriate to the cell line.

3. Replace the medium and apply fresh selection antibiotics every 4–7 days (approximately two times per week).

4. Stable colonies form within 1–2 weeks. Cells from the negative DNA control dish die off.

β-GALACTOSIDASE ACTIVITY TEST AS A TRANSFECTION EFFICIENCY CONTROL

Harvesting the Transfected Adherent Cells

1. Gently wash the transfected cells 1–2 times with PBS depending on the cell adherence to the tissue culture dish.

2. Add 1 ml of lysis buffer directly to the tissue culture dish and place at –20°C until frozen.

3. Thaw the tissue culture dish at room temperature and transfer the cell extract to a 1.7-ml microcentrifuge tube.

4. Spin the tube in a microcentrifuge at 12,000 × *g* for 5 minutes to pellet the cell debris.

5. Transfer the supernatant to a fresh microcentrifuge tube and store at –20°C or proceed to *β–Galactosidase Assay*.

Harvesting the Transfected Suspended Cells

1. Transfer the transfected cells into 15-ml centrifuge tubes and spin for 5 minutes at 200 × *g* to pellet the cells.

2. Remove and discard the supernatant, add 1 ml of PBS to the cell pellet, and transfer the resulting cell suspension to a 1.7-ml microcentrifuge tube.

3. Spin the tube in a microcentrifuge at 200 × *g* for 5 minutes to pellet the cells.

4. Remove and discard the supernatant, add 1 ml of lysis buffer to the cell pellet, and place at –20°C until frozen.

5. Thaw the cell extract and spin the tube in a microcentrifuge at 12,000 × *g* for 5 minutes to pellet the cell debris.

6. Transfer the supernatant to a new microcentrifuge tube and store at –20°C or proceed to *β–Galactosidase Assay*.

β-GALACTOSIDASE ASSAY

Notes *We recommend performing the β-galactosidase assay with the same reagents as are supplied in the β-Galactosidase Assay Kit.*

Add fresh β-mercaptoethanol to buffer A[§] prior to the β-galactosidase assay.

1. Add 100 µl of transfected cell extract to 900 µl of buffer A.
2. Incubate for 5 minutes at 37°C.
3. Add 200 µl of ONGP substrate solution,[§] vortex, and begin timing.
4. Incubate at 37°C, monitoring the reaction until a bright yellow color develops.

   Note *Incubation times for color development will vary. (The accuracy of the color reaction diminishes after ~45 minutes.)*

5. Terminate the color reaction by adding 500 µl of stop solution (1 M Na_2CO_3) and record the incubation time from step 4 to use in calculating the enzyme activity.
6. Read the optical density at OD420. (The optimum OD420 is between 0.6 and 0.9.)

β-GALACTOSIDASE HISTOCHEMICAL STAINING ASSAY

1. Wash the transfected cells twice with 3 ml of PBS.
2. Fix the cells for 5 minutes at 4°C by adding 5 ml of fixing solution.[§]
3. Wash the cells once with 5 ml of PBS.
4. Add 3–5 ml of histochemical staining solution[§] to the tissue culture dish.
5. Incubate the cells at 37°C for 14–24 hours.
6. Wash the cells twice with PBS and then add ~2 ml of PBS to cover the cells.

   Note *The use of fluorescein substrates such as fluorescein di-(β-galactopyranoside) allows for a more sensitive method of estimating percent of transfected cells.*[1]

[§] See *Preparation of Media and Reagents*.

TROUBLESHOOTING

Observation	Suggestion(s)
The transfection efficiency is low	Optimize the amount of the LipoTAXI transfection reagent between 15 and 60 µl for each cell type
	Optimize the amount of the plasmid DNA used for each cell type
	Optimize the incubation time of the transfection reaction for each cell type between 3 and 10 hours
	Ensure that serum was not present during the initial complex formation step
	Ensure that the LipoTAXI transfection reagent has not been frozen; store the reagent at room temperature
	Ensure the cells are 60–80% confluent at the time of transfection
	Ensure the plasmid DNA has an $OD_{260/280}$ ratio of ~ 1.8–2.0 and is endotoxin free
The reporter assay is problematic	Perform a positive control for the transfection assay
	The promoter element of the transfected DNA may not be recognized in the cell type used. Ensure that the promoter element used is expressed in the cell type used
The incidence of cell toxicity is high	Using too much LipoTAXI transfection reagent causes cell toxicity. Perform a dose–response curve varying the amount of transfection reagent while maintaining the amount of DNA
	Perform a dose–response curve varying the amount of DNA while maintaining the amount of transfection reagent
	Decrease the incubation time of the DNA–transfection reagent complex with the cells
	Increase the density of the cells used
	Cell viability may decrease in the absence of serum Limit the washes in serum-free medium or add a minimal serum concentration to the transfection system
	Do not add antibiotics to the media during the transfection
	Antibiotics may have been added too soon after transfection (for stable transfection experiments). Allow at least 48 hours for cells to express resistance genes before adding selection antibiotics

PREPARATION OF MEDIA AND REAGENTS

Buffer A

100 mM sodium phosphate (pH 7.5)
10 mM KCl
1 mM $MgSO_4$
50 mM β-mercaptoethanol (add just before use)

Fixing Solution

Note *Handle formaldehyde under a fume hood*

2% formaldehyde
0.2% gluteraldehyde
1× PBS
Sterile water to the desired final volume

Histochemical Reaction Mixture

3 mM potassium ferricyanide $[K_3Fe(Cn)_6]$
3 mM potassium ferrocyanide $[K_4Fe(Cn)_6]$
10% DMSO or DMF
2 mM $MgCl_2$
1× PBS
1 mg/ml X-gal (add just before use)
Sterile water to the desired final volume

PBS (Phosphate-Buffered Saline)

137 mM NaCl
2.6 mM KCl
10 mM Na_2HPO_4
1.8 mM KH_2PO_4
Adjust the pH to 7.4 with HCl

ONGP Substrate Solution

4 mg/ml in 100 mM sodium phosphate (pH 7.5)

Lysis Buffer

0.25 M Tris-HCl (pH 7.8)
0.5% Nonidet P-40 (NP-40)

X-gal Stock Solution

40 mg/ml in DMSO

REFERENCE

1. Bronstein, I., Fortin, J., Stanley, P. E., Stewart, G. S. and Kricka, L. J. (1994) *Anal Biochem* 219(2):169–81.

ENDNOTES

LipoTAXI® is a registered trademark of Genetic Applications LLC.

MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.

STRATAGENE

An Agilent Technologies Division

LipoTAXI® Mammalian Transfection Kit

Catalog #204110

QUICK-REFERENCE PROTOCOL

Transfecting Adherent Cells with the LipoTAXI® Mammalian Transfection Kit

- Inoculate a 60-mm tissue culture dish with 4×10^5–11×10^5 exponentially growing cells and grow overnight in 5 ml of the appropriate medium
- Transfer 900 µl of DMEM–SA to a polystyrene tube, add 35–100 µl of LipoTAXI transfection reagent, and mix the transformation mixture
- Add either 7 µg of the control plasmid or 5–10 µg (0.1–1.0 µg/µl) of the experimental DNA and, if performing a stable transfection, prepare a negative DNA control
- Mix gently (do not vortex) and allow to complex for 15–30 minutes at room temperature
- Aspirate the medium from the tissue culture dish
- Add 1.5 ml of DMEM (serum optional) to the transfection mixture and then transfer this entire mixture dropwise to the tissue culture dish while swirling
- Incubate for 4–6 hours in a humidified incubator at 37°C under 5% CO_2
- Add 2.5 ml of DMEM+S at twice the normal serum concentration and incubate overnight
- Replace the medium with 5.0 ml of fresh, complete medium
- Incubate for 24–72 hours or proceed to the next step if performing a stable transfection
- Split the cells to the desired ratio (at least 1:10) and incubate overnight
- Apply selection antibiotics dropwise, swirling between drops, at an appropriate concentration
- Replace the medium and apply fresh antibiotics every 4–7 days (approximately two times per week)
- Stable colonies form within 1–2 weeks, while cells from the negative DNA control die off

Transfecting Suspension Cells with the LipoTAXI Mammalian Transfection Kit

- Wash the suspended cells in DMEM–SA and seed the cells at a density of 4×10^6–10×10^6 cells per 60-mm dish in 700 µl of DMEM–SA
- Transfer 900 µl of DMEM–SA to a polystyrene tube, add 35–100 µl of LipoTAXI transfection reagent, and mix the transformation mixture
- Add either 10 µg of control plasmid or 7–15 µg (0.1–1.0 µg/µl) of the experimental DNA and, if performing a stable transfection, prepare a negative DNA control
- Mix gently (do not vortex) and allow to complex for 15–30 minutes at room temperature
- Add 800 µl of DMEM (serum optional) to the transfection mixture and then transfer this entire mixture dropwise to the tissue culture dish while swirling
- Incubate for 4–6 hours in a humidified incubator at 37°C under 5% CO_2
- Add 3 ml of fresh, complete DMEM+S at twice the normal serum concentration
- Incubate for 24–72 hours or proceed to the next step if performing a stable transfection
- After 48 hours, seed a fresh tissue culture dish at one-third the density of the transfected cells
- Apply selection antibiotics dropwise, swirling between drops, at an appropriate concentration
- Replace the medium and apply fresh antibiotics every 4–7 days (approximately two times per week)
- Stable transformants survive after 1–2 weeks, while cells from the negative DNA control die off