

Transfectam[®] Reagent for the Transfection of Eukaryotic Cells

INSTRUCTIONS FOR USE OF PRODUCTS E1231 AND E1232.

Transfectam[®] Reagent for the Transfection of Eukaryotic Cells

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1. Description.....	1
2. Product Components and Storage Conditions	3
3. Recommended Assay Protocol For Medium	
Without Serum (60mm Plate)	3
A. Plating Cells for Transfection	3
B. Preparation of Transfectam [®] Reagent Stock	4
C. Transfection Protocol	5
4. Recommended Assay Protocol for Medium	
With Serum (60mm Plate)	6
A. Plating Cells for Transfection	6
B. Preparation of Transfectam [®] Reagent Stock	6
C. Transfection Protocol	6
5. Optimization of Transfection Efficiency	7
6. Troubleshooting.....	8
7. References	8
8. Related Products	9

1. Description

The ability to introduce DNA into cultured cells has provided a powerful means to study the function and control of mammalian genes. Commonly used techniques for gene transfer are calcium phosphate, DEAE-Dextran, electroporation and liposome-mediated transfer.

Transfectam[®] Reagent for the Transfection of Eukaryotic Cells^(a) is dioctadecylamidoglycyl spermine (DOGS), a synthetic, cationic lipopolyamine molecule. The spermine group is covalently attached through a peptide bond to the lipid moiety (Figure 1). The strong positive charge contributed by the spermine headgroup gives the molecule a high affinity for DNA (10^5 - 10^6 M⁻¹), coating the DNA with a cationic lipid layer, which facilitates binding to the cell membrane.



1. Description (continued)

Transfectam® Reagent allows efficient transfection of a wide range of eukaryotic cells (1-4). Transfectam® Reagent has been used for both stable and transient transfections, with both established cell lines and primary cell cultures and for in vivo applications (5,6).

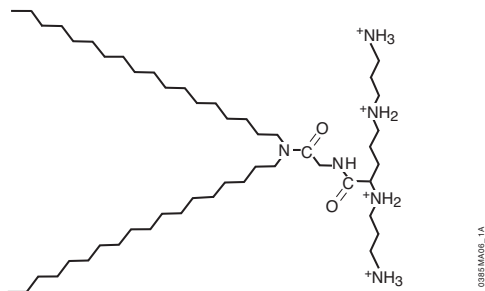


Figure 1. Structure of Transfectam® Reagent.

Citations using the Transfectam® Reagent

- Kisich, K.O., Malone, R.W., Feldstein, P.A. and Erickson, K.L. (1999) Specific inhibition of macrophage TNF- α expression by in vivo ribozyme treatment. *J. Immunol.* **163**, 2008-16.

Ribozymes designed to cleave TNF- α cDNA were cloned into the pGEM®-3Z Vector before being transcribed in vitro. The transcribed ribozymes then were mixed with Transfectam® Reagent and injected into mice intraperitoneally. Tissues and cells isolated from the mice were assayed at later time points for fluorescent or radiolabeled ribozymes. In vitro kinetic assays were also performed with ribozymes and RNA substrates labeled with T4 polynucleotide kinase.

- O'Rourke, J.P., Newbound, G.C., Hutt, J.A. and DeWille, J. (1999) CCAAT/enhancer-binding protein delta regulates mammary epithelial cell G0 growth arrest and apoptosis. *J. Biol. Chem.* **274**, 165829.

The authors investigated the effect of altered C/EBP δ content on G0 growth arrest and apoptosis in mammary epithelial cell lines. The pGEM®-4Z cloning vector was used in a cloning strategy to create an RNA antisense plasmid. For overexpression, HC11 cells were transfected with an overexpression plasmid using the Transfectam® Reagent. Cell proliferation of HC11, HC11/antisense and HC11/overexpression cell lines was assessed using the CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay.

For additional peer-reviewed articles that cite use of the Transfectam® Reagent, visit www.promega.com/citations/

2. Product Components and Storage Conditions

Product	Size	Cat. #
Transfectam® Reagent for the Transfection of Eukaryotic Cells	1mg	E1231

Includes sufficient reagent to transfect 80-260 μ g of DNA.

Product	Size	Cat. #
Transfectam® Reagent for the Transfection of Eukaryotic Cells	0.5mg	E1232

Includes sufficient reagent to transfect 40-130 μ g of DNA.

Storage Conditions: The Transfectam® Reagent is supplied as a trifluoroacetate salt (F.W. = 1,263) in dry form, which is stable at room temperature. After solubilization, store the Transfectam® Reagent at 4°C, where it is stable for at least 6 months.

Packaging: The Transfectam® Reagent is supplied in a glass ampule. The reagent is shipped at ambient temperature and packaged in a small box for storage.

3. Recommended Assay Protocol For Medium Without Serum (60mm Plate)

We recommend using medium with no added serum for transfection, as described below. Some components in serum may degrade the Transfectam® Reagent. The presence of albumin, heparin, trypsin or EDTA in the medium will decrease transfection efficiency.

! If cell viability is low in medium without serum, use the alternative protocol provided in Section 4.

Materials to Be Supplied by the User

- 100% ethanol (dehydrated)
- cell culture medium appropriate for the cell type used: with and without serum

3.A. Plating Cells for Transfection

Plate cells the day before transfection. The plating density for any particular cell line will depend upon the growth rate. Adherent cells should be at 50-70% confluency the day of transfection. A general guideline is to plate about 5×10^5 cells per 60mm culture dish. Scale the number of cells up or down proportionately if using different size plates (see Table 1). Immediately before transfection, if cells were grown in medium with serum, gently wash the cells in serum-free medium and add 0.5ml of serum-free medium per dish.

Note: For more information about setting up transfection reactions, refer to www.promega.com/transfectionass1/

3.A. Plating Cells for Transfection (continued)

Suspension cells can be transfected using the following protocol and the equivalent of 10^6 suspended cells per assay. The volume of reagents can be scaled up or down proportionately, depending on the number of cells used per assay.

Table 1. Area of Culture Plates for Cell Growth.

Size of Plate	Growth Area (cm ²) ^a	Relative Area ^b
96-well	0.32	0.02X
24-well	1.88	0.09X
12-well	3.83	0.18X
6-well	9.4	0.45X
35mm	8.0	0.38X
60mm	21	1.00X
100mm	55	2.62X

^a This information is for Corning® culture dishes.

^b Relative area is expressed as a factor of the growth area of the 60mm dish. To determine the approximate plating density, multiply 5×10^5 cells by this factor. To determine the reagent volumes needed for plates other than 60mm plates, multiply the volumes by the appropriate "Relative Area" factor.

3.B. Preparation of Transfectam® Reagent Stock

1. Transfectam® Reagent is supplied in two sizes: 0.5mg and 1mg. Dissolve Transfectam® Reagent in 100% ethanol (dehydrated) with vortexing, and incubate at room temperature for at least 5 minutes. (Use 200µl of ethanol for the 0.5mg size; use 400µl of ethanol for the 1mg size [final concentration = 2mM]). Overnight storage at 4°C ensures complete solubilization and may give improved transfection efficiencies.

Note: For some cells, it may be desirable to minimize the ethanol concentration applied. If so, Transfectam® Reagent may be dissolved with vortexing in as little as 1/10 volume of ethanol (dehydrated), incubated at room temperature for 5 minutes, then further diluted to working concentration in water.

2. Mix the solution before each use. Store the remaining stock at 4°C, where it is stable for up to 6 months.

3.C. Transfection Protocol

The reagent volumes in this protocol are based on the use of 60mm culture dishes. Scale the reagent volumes up or down proportionately if using different size plates (see Table 1).

1. Add 1-5µg of plasmid DNA to 500µl of serum-free medium in a sterile tube, and vortex (Solution A). We recommend 5µg per 60mm dish for the initial tests with each DNA and cell source.
2. For each microgram of plasmid DNA used in Solution A, add 1.5-5µl of Transfectam® Reagent to 500µl of serum-free medium in a sterile tube, and mix (Solution B). For the initial tests, use 10µl of Transfectam® Reagent per 60mm dish (see Section 5).
3. Immediately mix Solutions A and B, and add directly to the cells prepared in Section 3.A. The final volume will be 1.5ml for a 60mm plate or per 10^6 suspended cells.
4. Incubate the cells and solution for 30 minutes to overnight. (Incubate for 2 hours for the initial tests.)
5. At the end of the incubation period, gently overlay cells with 4ml of complete medium with serum (37°C). It is not necessary to remove the transfection medium containing the Transfectam® Reagent/DNA mixture. Return the cells to the incubator, and continue the incubation for the appropriate length of time before analysis. For many reporter assays (luciferase, chloramphenicol acetyltransferase [CAT], β-galactosidase), 48 hours after DNA addition is sufficient.
6. Check the transfection efficiency using the appropriate reporter assay.

4. Recommended Assay Protocol for Medium With Serum (60mm Plate)

Materials to Be Supplied by the User

- 100% ethanol (dehydrated)
- 0.15M NaCl (sterile)
- complete medium containing serum

4.A. Plating Cells for Transfection

Plate cells the day before transfection. The plating density for any particular cell line will depend upon growth rate. Adherent cells should be 50-70% confluent on the day of transfection. A general guideline is to plate about 5×10^5 cells per 60mm culture dish. Scale the number of cells up or down proportionately for different size plates (see Table 1). Immediately before transfection, remove the medium from the cells and add 1.5ml of fresh medium.

Note: For more information about setting up transfection reactions, refer to: www.promega.com/transfectionasst/

Suspension cells can be transfected using the following protocol and the equivalent of 10^6 suspended cells per assay. The volume of reagents can be scaled up or down proportionately depending on the number of cells used per assay.

4.B. Preparation of Transfectam® Reagent Stock

1. Transfectam® Reagent is supplied in two sizes, 0.5mg and 1mg. Dissolve Transfectam® Reagent in 100% ethanol (dehydrated) with vortexing, and incubate at room temperature for at least 5 minutes. (Use 200µl of ethanol for the 0.5mg size; use 400µl of ethanol for the 1mg size [final concentration = 2mM]). Overnight storage at 4°C ensures complete solubilization and may give improved transfection efficiencies.

Note: For some cells, it may be desirable to minimize the ethanol concentration applied. If so, Transfectam® Reagent may be dissolved with vortexing in as little as 1/10 volume of ethanol (dehydrated), incubated at room temperature for 5 minutes, then further diluted to working concentration in water.

2. Mix the solution before each use. Store the remaining stock at 4°C, where it is stable for up to 6 months.

4.C. Transfection Protocol

The reagent volumes in this protocol are based on the use of 60mm culture dishes. Scale the reagent volumes up or down proportionately if using different size plates (see Table 1).

1. Add 1-5µg of plasmid DNA to 50µl of 150mM NaCl solution in a sterile tube, and vortex (Solution A). We recommend 5µg per 60mm dish for the initial tests with each DNA and cell source.

2. For each microgram of plasmid DNA used in Solution A, add 1.5-5µl of Transfectam® Reagent to 50µl of 150mM NaCl solution in a sterile tube, and mix (Solution B). For the initial tests, use 10µl of Transfectam® Reagent per 60mm dish (see Section 5).
3. Immediately mix Solutions A and B, wait 10 minutes, then add to the cells prepared in Section 4.A above.
4. Incubate cells with the solution for 30 minutes to overnight. (Incubate for 2 hours for the initial tests.)
5. At the end of the incubation period, gently overlay cells with 4ml of complete medium (37°C). It is not necessary to remove the transfection medium containing the Transfectam® Reagent/DNA mixture. Return cells to the incubator, and continue the incubation for the appropriate length of time before analysis. For many reporter assays (luciferase, chloramphenicol acetyltransferase [CAT], β-galactosidase), 48 hours after DNA addition is sufficient.
6. Check the transfection efficiency using the appropriate reporter assay.

5. Optimization of Transfection Efficiency

Follow these recommendations to obtain the best results possible:

- Optimize the volume/weight ratio of Transfectam® Reagent/DNA in the range of 1.5-5µl/µg DNA. A good initial test for a 60mm plate is 10µl of Transfectam® Reagent stock to 5µg DNA.
- Optimize the amount of DNA used in the range of 1-10µg DNA. It may not be necessary to increase the quantity of DNA significantly to obtain optimal results. In fact, if the first transfection results are satisfactory, a reduced DNA quantity can be tested (while keeping the optimal Transfectam® Reagent/DNA ratio constant).
- The transfection time depends on the specific DNA and cell system used and should be optimized between 30 minutes and overnight. It may be necessary to monitor cell viability if using serum-free medium for a prolonged period because some cells do not thrive under this condition.
- Calibrate the system using a test plasmid with reporter gene function. An ideal reporter gene product is not endogenous to the cell, can be expressed from a plasmid DNA and is usually an enzyme that can be conveniently assayed. Commonly used reporter genes are luciferase, chloramphenicol acetyltransferase (CAT) and β-galactosidase.

6. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: www.promega.com. E-mail: techserv@promega.com

Symptoms	Causes and Comments
No transfection or low transfection efficiency	Poor-quality DNA. The DNA should be purified on CsCl gradients or equivalent methods. The A_{260}/A_{280} of the DNA should be 1.8-1.9. Excessive cell death: <ul style="list-style-type: none"> Decrease the time of exposure of cells to the reagent. Lower the amount of input DNA and Transfectam® Reagent while holding the ratio constant. Remove the Transfectam®/DNA mixture from the cells after the transfection period and prior to adding complete medium. Test for transfection in the presence of serum.
Variable transfection efficiencies in replicate experiments	Suboptimal growth of cells: <ul style="list-style-type: none"> Check that cultures are Mycoplasma-free. Use cultured cells at low passage number. Variable cell density. Maintain a consistent state of confluency at the time of transfection for each experiment.

7. References

- Behr, J.P. *et al.* (1989) Efficient gene transfer into mammalian primary endocrine cells with lipopolyamine-coated DNA. *Proc. Natl. Acad. Sci. USA* **86**, 6982-6.
- Loeffler, J.P. *et al.* (1990) Lipopolyamine-mediated transfection allows gene expression studies in primary neuronal cells. *J. Neurochem.* **54**, 1812-5.
- Barthel, F. *et al.* (1993) Gene transfer optimization with lipospermine-coated DNA. *DNA and Cell Biology* **12**, 553-60.
- Remy, J.S. *et al.* (1994) Gene transfer with a series of lipophilic DNA-binding molecules. *Bioconjug. Chem.* **5**, 647-54.
- Demeneix, B.A. *et al.* (1994) Temporal and spatial expression of lipospermine-compacted genes transferred into chick embryos in vivo. *BioTechniques* **16**, 496-501.
- Tsukamoto, M. *et al.* (1995) Gene transfer and expression in progeny after intravenous DNA injection into pregnant mice. *Nature Genetics* **9**, 243-8.

8. Related Products

Transfection Reagents

Product	Size	Cat.#
TransFast™ Reagent	1.2mg	E2431
Tfx™-20 Reagent	4.8mg	E2391
Tfx™-50 Reagent	2.1mg	E1811
Tfx™ Reagents Transfection Trio	5.4mg	E2400

Tfx™ Reagents Transfection Trio contains one vial each of Tfx™-10, Tfx™-20 and Tfx™-50 Reagents. Each system contains sufficient reagent to transfect 200µg of DNA (at a 4:1 Tfx™ Reagent:DNA ratio).

pGL4 Luciferase Reporter Vectors

Please visit www.promega.com to see a complete listing of our reporter vectors.

Vector	Multiple Cloning Region	Reporter Gene	Protein Degradation Sequence	Reporter Gene Promoter	Mammalian Selectable Marker	Cat.#
pGL4.10[luc2]	Yes	luc2 ^A	No	No	No	E6651
pGL4.11[luc2P]	Yes	"	hPEST	No	No	E6661
pGL4.12[luc2CP]	Yes	"	hCL1-hPEST	No	No	E6671
pGL4.13[luc2/SV40]	No	"	No	SV40	No	E6681
pGL4.14[luc2/Hygro]	Yes	"	No	No	Hygro	E6691
pGL4.15[luc2P/Hygro]	Yes	"	hPEST	No	Hygro	E6701
pGL4.16[luc2CP/Hygro]	Yes	"	hCL1-hPEST	No	Hygro	E6711
pGL4.17[luc2/Neo]	Yes	"	No	No	Neo	E6721
pGL4.18[luc2P/Neo]	Yes	"	hPEST	No	Neo	E6731
pGL4.19[luc2CP/Neo]	Yes	"	hCL1-hPEST	No	Neo	E6741
pGL4.20[luc2/Puro]	Yes	"	No	No	Puro	E6751
pGL4.21[luc2P/Puro]	Yes	"	hPEST	No	Puro	E6761
pGL4.22[luc2CP/Puro]	Yes	"	hCL1-hPEST	No	Puro	E6771
pGL4.70[hRluc]	Yes	hRluc ^B	No	No	No	E6881
pGL4.71[hRlucP]	Yes	"	hPEST	No	No	E6891
pGL4.72[hRlucCP]	Yes	"	hCL1-hPEST	No	No	E6901
pGL4.73[hRluc/SV40]	No	"	No	SV40	No	E6911
pGL4.74[hRluc/TK]	No	"	No	HSV-TK	No	E6921
pGL4.75[hRluc/CMV]	No	"	No	CMV	No	E6931
pGL4.76[hRluc/Hygro]	Yes	"	No	No	Hygro	E6941
pGL4.77[hRlucP/Hygro]	Yes	"	hPEST	No	Hygro	E6951
pGL4.78[hRlucCP/Hygro]	Yes	"	hCL1-hPEST	No	Hygro	E6961
pGL4.79[hRluc/Neo]	Yes	"	No	No	Neo	E6971
pGL4.80[hRlucP/Neo]	Yes	"	hPEST	No	Neo	E6981
pGL4.81[hRlucCP/Neo]	Yes	"	hCL1-hPEST	No	Neo	E6991
pGL4.82[hRluc/Puro]	Yes	"	No	No	Puro	E7501
pGL4.83[hRlucP/Puro]	Yes	"	hPEST	No	Puro	E7511
pGL4.84[hRlucCP/Puro]	Yes	"	hCL1-hPEST	No	Puro	E7521

^Aluc2 = synthetic firefly luciferase gene. ^BhRluc = synthetic *Renilla* luciferase gene.

8. Related Products (continued)

Luciferase Assays

Product	Size	Cat.#
Dual-Glo™ Luciferase Assay System	10ml	E2920
	100ml	E2940
	10 × 100ml	E2980
Bright-Glo™ Luciferase Assay System	10ml	E2610
	100ml	E2620
Steady-Glo	10ml	E2510
	100ml	E2520
	10 × 100ml	E2550
EnduRen™ Live Cell Substrate	0.34mg	E6481
	3.4mg	E6482
	34mg	E6485
Luciferase Assay System	100 assays	E1500
Luciferase Assay System, 10-pack	1,000 assays	E1501
Luciferase 1000 Assay System	1,000 assays	E4550
Luciferase Assay Reagent	1,000 assays	E1483
Renilla Luciferase Assay System	100 assays	E2810
	1,000 assays	E2820
Dual-Luciferase® Reporter Assay System	100 assays	E1910
Dual-Luciferase® Reporter Assay System 10-pack	1,000 assays	E1960
Dual-Luciferase® Reporter 1000 Assay System	1,000 assays	E1980
ViviRen™ Live Cell Substrate	0.37mg	E6491
	3.7mg	E6492
	37mg	E6495

β-Galactosidase Vector Reagents

Product	Size	Cat.#
pSV-β-Galactosidase Control Vector	20µg	E1081
β-Galactosidase Enzyme Assay System With Reporter Lysis Buffer	65 assays	E2000
	Reporter Lysis 5X Buffer	30ml
Beta-Glo Assay System	10ml	E4720
	100ml	E4740
	10 × 100ml	E4780

Plasmid DNA Purification System

Product	Size	Cat.#
PureYield™ Plasmid Midiprep System	25 preps	A2492
	100 preps	A2495
PureYield™ Plasmid Maxiprep System	10 preps	A2392
	25 preps	A2393

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