GenePORTER™ 2 Transfection Reagent

Product Summary

Cat. No: T202007

Description: GenePORTER 2 transfection reagent is a unique formulation of the neutral lipid dioleoyl phosphatidylethanolamine (DOPE) and a proprietary cationic lipid derived from our innovative direct hydrophilic conjugation (DHC) technology. Each GenePORTER 2 reagent kit includes two special DNA diluent buffers that increase transfection efficiency in the presence of serum. One vial of GenePORTER 2 reagent (0.75 ml) is sufficient for 75 transfections using 2 µg of DNA per well and at least 100 transfections with the New DNA diluent B. GenePORTER 2 reagent is suitable for in vitro and in vivo transfection studies.

Components: Dried GenePORTER 2 lipids film (one vial)
GenePORTER 2 transfection-grade hydration buffer (one vial, 0.8 ml)
DNA diluent (two vials, 2 ml per vial)
DNA diluent B (one vial, 4 ml)

Storage: Store components at 4°C.

Stability: Dried GenePORTER 2 reagent is stable for 1 year at 4°C.
Hydrated GenePORTER 2 reagent is stable for 6 months at 4°C.
DNA diluent and DNA diluent B are stable for 6 months at 4°C.

Usage: See page 2 for Recommended Uses for DNA Diluents
Follow the protocol from page 3 to 5 for DNA Diluent.
Follow the protocol from page 6 to 7 for DNA Diluent B.

INTRODUCTION

GenePORTER 2 transfection reagent is the newest advance in gene delivery developed by Gene Therapy Systems. While featuring all of the advantages of DHC technology as the original GenePORTER reagent, GenePORTER 2 reagent delivers higher expression levels than other commercially available products. With the two optimized DNA diluent buffers, GenePORTER 2 performs over a broad range of cell types and, importantly, in the presence of serum.

- Highest efficiencies in diverse cell types
- Excellent performance in presence of serum
- Extended shelf life
- Same DHC technology as the original GenePORTER reagent

Table 1: Transfected Cell Types
GenePORTER 2 reagent was successfully used to transfect β-galactosidase reporter gene into the cell lines listed.

Transfected Cell Types	
HeLa S3	BHK-21
293	CHO-K1
MDCK	CV1
NIH 3T3	COS-1
B16-F0	COS-7
PC-12	HepG2
K562	P19
HeLa	HUVEC-C

Voice: (858) 457-1919 Fax Lines: (858) 623-9494 • (858) 587-1499
Website: http:/www.genetherapysystems.com

RECOMMENDED USES FOR DNA DILUENTS

Select the DNA diluent most suitable for the cell types listed below. If your cell type is not listed, choose the cell type that closely matches yours.

Cell Lines	DNA Diluent	DNA Diluent B	Serum
HeLa-S3	★	★★	○
HeLa	★	★★	○
COS-1	★	★	●
COS-7	★	★	●
Hep-G2	★	★	●
NIH-3T3	★	★	●
MDCK	★	★★	○
K-562	★	★★	○
CV-1	★	★	●
B15-F0	★	★	●
293	★	★	●
BHK-21	★	★	●
CHO-K1	★▲	★▲	●
PC-12	★	NR	●
P19	★	★	●
HUVEC-C	★	★	●
Jurkat	◆	◆	○

LEGEND:

★	Works well
★★	Works better
○	Works well without serum
●	Works well with and without serum
NR	Not recommended
▲	Highest levels of expression are obtained without serum during the first hour of transfection
◆	Original GenePORTER reagent is recommended.

10190 Telesis Court, San Diego, CA 92121
Voice: (858) 457-1919 Fax Lines: (858) 623-9494 • (858) 587-1499
Website: http:/www.genetherapysystems.com

PROTOCOL FOR DNA DILUENT

Direction

- Hydrate GenePORTER 2 lipid film at room temperature with 0.75 ml of the hydration buffer. Vortex for 10 seconds at top speed before use. Store the hydrated reagent at 4°C and vortex briefly before each use.
- Use the DNA diluent to prepare the DNA solution. Use 25 µl of diluent for 1 µg DNA. Avoid vortexing the DNA diluent solution.
- For most cell types, use 5 µl of GenePORTER 2 reagent with 1 µg of DNA.

Transfection of adherent cells

1. Dilute the hydrated GenePORTER 2 reagent with serum-free medium.
   - Refer to Table 2 for the appropriate volume of serum-free medium.

2. Dilute the DNA with the DNA diluent and incubate 1 to 5 minutes at room temperature. Do not incubate longer than 5 minutes.
   - Refer to Table 2 for the appropriate volume of DNA diluent.
   - Avoid vortexing the DNA diluent.

3. Add the DNA solution to the diluted GenePORTER 2 reagent. Incubate at room temperature for 5 to 10 minutes to form GenePORTER 2/DNA complexes (lipoplexes).
   - Do not incubate longer than 30 minutes

4. Add the mixture of GenePORTER 2/DNA complexes directly to the cells growing in serum-containing culture medium.[a, b] Incubate at 37°C.
   - Refer to Table 3 for appropriate transfection volumes.

5. 24 hours post transfection, add fresh growth media as needed.[c] Depending on the cell type and promoter activity, the assay for the reporter gene can be performed 24 to72 hours following transfection.[d]

Table 2

Volumes of Transfection Reagents [e]

DNA (µg)	DNA diluent (provided) (µl)	Gene-PORTER2 (µl)	Gene-PORTER2 diluent (Serum free medium) (µl)
0.5	12.5	2.5	10
1	25	5	20
2	50	10	40
4	100	20	80
8	200	40	160

Table 3

Transfection Volumes and DNA Amount for Various Culture Dishes [e]

Tissue Culture Dish	DNA (µg)	Transfection Volume (ml)
96-well	0.1-0.5	0.1
24-well	0.5-2	0.25
6-well	2-6	1
60 mm	6-8	2.5
100 mm	8-12	5

10190 Telesis Court, San Diego, CA 92121
Voice: (858) 457-1919 Fax Lines: (858) 623-9494 • (858) 587-1499
Website: http:/www.genetherapysystems.com

Notes:

[a]Cells plated the day before transfection should be 50% to 70% confluent on the day of transfection. Omitting antibiotics from the media during transfection may increase expression levels; this effect is cell-type dependent and usually small.

[b]For some cells (such as HeLa S3, MDCK, CHO-K1), higher transfection efficiencies can be achieved when the initial 4-hour incubation is done in serum-free media. After this step, add one volume of medium containing 20% serum, then proceed as in Step 5.

[c]For some cell types, the old media can be replaced with fresh media at this step.

[d]The same protocol can be used to produce stably transfected cells: 48 to 72 hours post transfection, put the cells in fresh medium containing the appropriate selection antibiotic. It is important to wait at least 48 hours before exposing the transfected cells to the selection media. For some cell types it may be necessary to wait as long as 4 to 5 days before applying the selection condition.

[e]Although GenePORTER 2 reagent consistently delivers high transfection efficiencies in a wide range of cell types, in order to obtain maximum efficiency in particular cell lines, some optimization may be needed. The two critical variables are the ratio of GenePORTER 2 reagent to DNA and the quantity of DNA. For optimization, first maintain a fixed ratio of GenePORTER 2 reagent to DNA, then vary the DNA quantity over the suggested range. If necessary, optimize the ratio of GenePORTER 2 reagent to DNA by using 3 to 6 µl of reagent for each 1 µg of DNA. Use a low DNA quantity to optimize this ratio. Following this process, cell number can also be optimized.

Transfection of suspension cells

Although GenePORTER 2 reagent works well for cells such as K562 and PC 12, which can grow in suspension, it does not work well for Jurkat cells. For transfection of Jurkat cells, we recommend using the original GenePORTER reagent.

For suspension cells, the protocol is the same as described for adherent cells, with the following exceptions:

1. The day before transfection, split the cells so they are in good condition on the day of transfection.

2. While the GenePORTER 2/DNA complexes are incubating, spin down the cells, resuspend them at 1 x 10^6 or 2 x 10^6 cells/ml in medium with or without serum, and transfer the appropriate volume to the dish (Table 4).

3. Prepare the mixture of GenePORTER 2/DNA complexes as above, add it directly to the cells, and mix well by gently pipetting 2 to 3 times.[f] Incubate at 37°C and proceed as described for adherent cells.[g]

Table 4

Suggested Conditions for Transfecting Suspension Cells [h]

Tissue Culture Dish	Number of Cells	Transfection Volume
96-well	1 x 10^5	0.1 ml
24 well	0.5 x 10^6	0.25 ml
6-well	2 x 10^6	1 ml
60 mm	5 x 10^6	2.5 ml
100 mm	1 x 10^7	5 ml

Notes:

[f]This step is important because some suspension cells have a tendency to clump, and the reagent does not easily access cells in the center of clumps. Gentle pipetting of cells disrupts these clumps and produces a true single-cell suspension, which will increase transfection efficiency.

[g]For some hematopoietic cell lines, mitogenic agents like PHA or PMA may be added to the cells 4 hours after transfection to a final concentration of 1 µg/ml or 50 ng/ml, respectively, to enhance the levels of gene expression.

[h]For suspension cells, the optimization procedure is the same as adherent cells (Table 3 and Notes[e]).

Optional protocol for low quantity DNA transfection

The following revised protocol[1, 2] can be used to facilitate pipetting and transfer of DNA/lipids complexes to the cells when a low quantity of DNA (≤ 1 µg) is used for the transfection.

1. Dilute hydrated GenePORTER 2 reagent with serum-free medium (Table 5).

2. First dilute the DNA diluent in serum-free medium and then add the DNA. See Table 5 for volumes of serum-free medium, DNA diluent, and DNA amount. Incubate 1 to 5 minutes at room temperature.

3. Proceed as in Steps 3 though 5 as described for adherent cells.

Table 5
Recommended Amounts of Reagents for Optional Protocol

A. Dilution of GenePORTER 2 Reagent

DNA (µg)	Serum-free medium (µl)	GenePORTER 2 Reagent (µl)
0.125	49.37	0.63
0.25	48.75	1.25
0.5	47.5	2.5
1	45	5

B. DNA Dilution

Serum-free medium (µl)	DNA diluent (µl)	**DNA (µg)**
46.8	3.12	**0.125**
43.75	6.25	**0.25**
37.5	12.5	**0.5**
25	25	**1**

C. Transfection Volume and DNA Amounts for Different Plates

Tissue Culture Dish	DNA (µg)	Transfection Volume (ml)
96-well	0.1-0.25	0.1
24-well	0.5-2	0.25

10190 Telesis Court, San Diego, CA 92121
Voice: (858) 457-1919 Fax Lines: (858) 623-9494 • (858) 587-1499
Website: http:/www.genetherapysystems.com

PROTOCOL FOR DNA DILUENT B

Direction

- Hydrate GenePORTER™ 2 lipid film at room temperature with 0.75 ml of the hydration buffer. Vortex for 10 seconds at top speed before use. Store the hydrated reagent at 4°C and vortex briefly before each use.
- Use the DNA diluent B to prepare the DNA solution. Use 25 µl of diluent B for 1 µg DNA.
- Use 3.5 µl of GenePORTER 2 reagent with 1 µg of DNA.

Transfection of adherent cells

1. Dilute the hydrated GenePORTER 2 reagent with serum-free medium.
   - Refer to Table 6 for the appropriate volume of serum-free medium.

2. Dilute the DNA with the New DNA diluent B, mix well by pipetting several times and incubate 5 minutes at room temperature.
   - Refer to Table 6 for the appropriate volume of New DNA diluent B.
   - Avoid vortexing the New DNA diluent B solution.

3. Add the DNA solution to the diluted GenePORTER 2 reagent. Incubate at room temperature for 5 minutes to form GenePORTER 2/DNA complexes (lipoplexes).
   - Do not incubate longer than 30 minutes.

4. Add the GenePORTER 2/DNA complexes directly to the cells that are in serum-containing or serum-free culture medium. [a, b] Incubate at 37°C (if serum-free medium is used refer to note b).
   - Refer to Table 7 for appropriate transfection volumes.

5. 24 hours post transfection, add fresh growth media as needed.[c] Depending on the cell type and promoter activity, the assay for the reporter gene can be performed 24 to72 hours following transfection.[d]

Table 6

Volumes of Transfection Reagents [e]

DNA (µg)	DNA diluent B (provided) (µl)	Gene-PORTER2 (µl)	Gene-PORTER2 diluent (Serum free medium) (µl)
0.5	12.5	1.75	10.75
1	25	3.5	21.5
2	50	7	43
4	100	14	86
8	200	28	172

Table 7

Transfection Volumes and DNA Amount for Various Culture Dishes [e]

Tissue Culture Dish	DNA (µg)	Transfection Volume (ml)
96-well	0.1-0.5	0.1
24-well	0.5-2	0.25
6-well	2-6	1
60 mm	6-8	2.5
100 mm	8-12	5

Voice: (858) 457-1919 Fax Lines: (858) 623-9494 • (858) 587-1499
Website: http:/www.genetherapysystems.com

Notes:

[a]Cells plated the day before transfection should be 50% to 70% confluent on the day of transfection.

[b]For some cells (such as HeLa-S3, MDCK or CHO-K1), higher transfection efficiencies can be achieved when the initial 4-hour incubation is done in serum-free media. After this step, add one volume of medium containing 20% serum, then proceed as in Step 5.

[c]For some cell types, the old media can be replaced with fresh media at this step.

[d]The same protocol can be used to produce stably transfected cells: 48 to 72 hours post transfection, put the cells in fresh medium containing the appropriate selection antibiotic. It is important to wait at least 48 hours before exposing the transfected cells to the selection media.

[e]To obtain maximum efficiency in particular cell lines, some optimization may be needed. The two critical variables are the ratio of GenePORTER 2 reagent to DNA and the quantity of DNA. First maintain a fixed ratio of GenePORTER 2 reagent to DNA, then vary the DNA quantity over the suggested range. If necessary, optimize the ratio of GenePORTER 2 reagent to DNA by using 2 to 6 µl of reagent for each 1 µg of DNA. Use a low DNA quantity to optimize this ratio. Following this process, cell number can also be optimized.

Transfection of suspension cells

For Jurkat cells, we recommend using the original GenePORTER reagent. The protocol for suspension cells is the same as described for adherent cells, with the following exceptions.

1. The day before transfection, split the cells so they are in good condition on the day of transfection.

2. While the GenePORTER 2/DNA complexes are incubating, spin down the cells, resuspend them at 1 x 10^6 or 2 x 10^6 cells/ml in medium with or without serum, and transfer the appropriate volume to the dish (Table 8).

3. Prepare the mixture of GenePORTER 2/DNA complexes as above, add it directly to the cells, and mix well by gently pipetting 2 to 3 times.[f] Incubate at 37°C and proceed as described for adherent cells.[g]

Table 8

Suggested Conditions for Transfecting Suspension Cells [h]

Tissue Culture Dish	Number of Cells	Transfection Volume
96-well	1 x 10^5	0.1 ml
24 well	0.5 x 10^6	0.25 ml
6-well	2 x 10^6	1 ml
60 mm	5 x 10^6	2.5 ml
100 mm	1 x 10^7	5 ml

Notes:

[f]This step is important because some suspension cells have a tendency to clump, and the reagent does not easily access cells in the center of clumps. Gentle pipetting of cells disrupts these clumps and produces a true single-cell suspension, which will increase transfection efficiency.

[g]For some hematopoietic cell lines, mitogenic agents like PHA or PMA may be added to the cells 4 hours after transfection to a final concentration of 1 µg/ml or 50 ng/ml, respectively, to enhance the levels of gene expression.

[h]For suspension cells, the optimization procedure is the same as adherent cells (Table 7 and Note[e]).

DETECTION OF EXPRESSED REPORTER GENES

b-Galactosidase

Gene Therapy Systems offers several β-galactosidase assay kits for your convenience. Alternatively, you may use the following procedure[1] to assay β-galactosidase activity:

Briefly, aspirate the culture media post transfection. Lyse the transfected cells from each well of a 96-well plate with 50 µl of the lysis buffer [0.1% Triton X-100 (w/v) in 250 mM Tris-HCl, pH 8.0], then subject the cells to one freeze-thaw cycle (freeze at -70°C and thaw at room temperature). While the cells are being lysed, prepare a β-galactosidase (*E.coli*; Sigma) standard curve with 0.5% BSA in PBS (w/v). Once the plate of lysed cells is completely thawed, transfer a 50-µl aliquot of each point on the standard curve to control wells of the plate. Typically, β-galactosidase expression ranges from 10,000 to 2,000,000 pg. Develop color by adding 150 µl of 1 mg/ml of chlorophenol red-β-D-galactopyranoside (CPRG; Boehringer Mannheim) dissolved in β-gal buffer (1 mM MgCl2; 10 mM KCl; 50 mM β-mercaptoethanol; and 60 mM Na_2HPO_4, pH 8.0). Allow the reaction to proceed at room temperature until the red color develops (2 min to 4 hours, depending on cell type). Read absorbance at 580 nm.

An immunohistochemical approach for quantifying β-galactosidase has also been reported.[3]

Green Fluorescent Protein

When green fluorescent protein (GFP) is the reporter gene used for transfections, use epifluorescence or confocal microscopy to detect expression. GFP has an excitation peak at 470 to 490 nm and emission peak at 510 nm. Expression levels of GFP can also be monitored by fluorescence-activated cell sorter analysis (FACS).[4]

Secreted Alkaline Phosphatase

When heat-stable secreted alkaline phosphatase (SEAP) is the reporter gene used for transfections, use the following assay. Heat supernatants from transfected cells at 65°C for 30 min to inactivate endogenous alkaline phosphatase activity. The SEAP transgene is stable during this treatment. Take aliquots of the culture media 48 hours posttransfection, and determine the SEAP activity quantitatively by using a colorimetric assay based on hydrolysis of the chromogenic substrate para-nitrophenyl phosphate (PNPP). Dissolve 1 mg/ml of PNPP reagent in a solution of 1 mM $MgCl_2$ and 100 mM diethanolamine, pH 9.8. Add 10 µl of 0.05% Zwittergent in PBS (free Ca^{2+} and Mg^{2+}) into each well of a 96-well plate. Then add 20 µl of the heated cell culture media to each well. For control wells, 20 µl of water is used to normalize the volume. An alkaline phosphatase standard (EIA grade calf intestine alkaline phosphatase; Boehringer Mannheim) can be used to generate a standard curve from 10 to 10,000 pg per well. Add 200 µl of the PNPP substrate to each well to start the enzymatic reaction. Allow the reaction to incubate at room temperature until the yellow color develops. Using 0.05% Zwittergent in PBS as the diluent virtually reduces the background to zero, which increases the detection limit of the assay. Read the plates at 405 nm using either kinetic or static mode.

REFERENCES

1. Felgner, JH et al. Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. *J Biol Chem.* 1994; 269:2550-2561.
2. Felgner, PL et al. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. *Proc Natl Acad Sci USA* 1987;84:7413-7417.
3. Gussoni, E et al. A method to co-detect introduced genes and their products in gene therapy protocols. *Nature Biotechnology*. 1996; 14:1012-1015.
4. Cheng, L et al. Use of green fluorescent protein variants to monitor gene transfer and expression in mammalian cells. *Nature Biotechnology*. 1996; 14:606-609.

Voice: (858) 457-1919 Fax Lines: (858) 623-9494 • (858) 587-1499
Website: http:/www.genetherapysystems.com

RELATED PRODUCTS

Product	Catalog #
GenePORTER™ Transfection Reagent	
150 reactions (1.5 ml)	T201015
750 reactions (5 x 1.5ml)	T201075
GenePORTER™ 2 Transfection Reagent	
150 reactions (1.5 ml)	T202015
750 reactions (5 x 1.5 ml)	T202075
GeneSilencer™ siRNA Transfection Reagent	
200 reactions (0.75 ml)	T500750
750 reactions (5 x 0.75 ml)	T505750
NeuroPORTER™ Transfection Reagent	
1.5 ml	T400150
5 x 1.5 ml	T400750
BioPORTER® Protein Delivery Reagent QuikEase Kit	
24 single use vials	BP502424
96 single use vials	BP509696
phCMV1 Expression Vector Kit	P003100
phCMV2 Expression Vector Kit	P003200
phCMV3 Expression Vector Kit	P003300
gWiz High-Expression Vectors:	
β-galactosidase vector	P010200
Luciferase vector	P030200
GFP vector	P040400
Secreted AP vector	P050200
Enhanced β-galactosidase Assay Kit (CPRG)	A10100K
β-galactosidase Assay Kit (ONPG)	A10200K
X-Gal Staining Assay Kit	A10300K

Please contact us for a complete list of our products or visit our website for more information.

10190 Telesis Court, San Diego, CA 92121
Voice: (858) 457-1919 Fax Lines: (858) 623-9494 • (858) 587-1499
Website: http:/www.genetherapysystems.com