A division of Gene Therapy Systems, Inc.

GenePORTER® 2 Transfection Reagent
QuikEase™ Single-Use Tubes

Product Summary

Cat. No: **T202096**

Description: GenePORTER 2 transfection reagent is a unique formulation of the neutral lipid dioleoyl phosphatidylethanolamine (DOPE) and a proprietary cationic lipid derived from the patented direct hydrophilic conjugation (DHC) technology. The GenePORTER 2 QuikEase Kit contains 96 single reaction tubes, which makes transfection easier and more convenient. Each tube contains sufficient GenePORTER 2 reagent for transfecting 6 μg or less of DNA.

Components: Dried GenePORTER 2 lipid film (96 tubes)
DNA diluent (6 ml)
DNA diluent B (8 ml)

Storage: Store components at 4°C.

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

INTRODUCTION

GenePORTER 2 transfection reagent is the newest advance in gene delivery developed by Genlantis. 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, especially in difficult-to-transfect cell lines. With the two optimized DNA diluents, GenePORTER 2 performs over a broad range of cell types with or without the presence of serum.

- Highest efficiencies in diverse cell types
- Best with difficult-to-transfect cell lines
- Excellent performance in presence of serum
- Convenient and easy protocols
- Extended shelf life

Examples of Transfected Cell Types

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

GenePORTER 2 reagent was successfully used to transfect β-galactosidase reporter gene into many cell lines. For a complete list, please visit our web site at www.genlantis.com.

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DIRECTIONS

- Use the DNA diluent to prepare the DNA solution. Choose appropriate diluent according to the following table. If your cell type is not listed, start with the DNA Diluent B.
- Hydrate GenePORTER 2 dry film in the tube with the DNA solution.
- Add more medium to the DNA/GenePORTER 2 complexes, transfer the complexes onto the cells.

RECOMMENDED USES FOR DNA DILUENTS

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.

EXAMPLE PROTOCOLS

Transfection of adherent cells (6-well plates)

1. Plate cells so that they will be 50-70% confluent on the day of transfection.
2. Dilute the 4 μg DNA with 100 μl DNA Diluent or 6 μg DNA with 150 μl DNA Diluent B. Incubate 5 minutes at room temperature.
3. Add serum-free medium to the diluted DNA to bring up the volume to 250μl.
4. Hydrate the dry GenePORTER 2 reagent with the DNA solution, pipette up and down 5 times. Incubate at room temperature for 10 - 20 minutes to form GenePORTER 2/DNA complexes (lipoplexes).
5. Add the GenePORTER 2/DNA complexes directly to the cells growing in serum-containing culture medium. The following table indicates the suggested volumes to use per well for various tissue culture plates. If using a tissue culture plate with wells smaller than those for 6-well plates, subdivide the lipoplex

volumes as indicated. Add the appropriate amount of medium to each well to bring the volume up to the total transfection volume indicated.

Table 1: Suggested Transfection Volumes and DNA Amounts

Tissue culture dish	Lipoplex volume/well	DNA amount/well	Total transfection volume/well
6-well	250 µl	4 µg	1 ml
12-well	125 µl	2 µg	500 µl
24-well	62.5 µl	1 µg	250 µl
96-well	31.25 µl	0.5 µg	100 µl

6. Incubate at 37°C.

7. 24 hours post transfection, add fresh growth media as needed.[b] Depending on the cell type and promoter activity, the assay for the reporter gene can be performed 24 to72 hours following transfection.[c]

Notes:

[a] 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.

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

[c] 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 antibiotics. 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.

Suggested DNA optimization ranges for different tissue culture plates:

- 6-well. Transfer 3 - 5 µg of DNA directly into each well and adjust the final volume to 1ml.
- 12-well. Transfer 1 - 3 µg of DNA directly into each well and adjust the final volume to 500µl.
- 24-well. Transfer 0.5 - 1.5 µg of DNA directly into each well and adjust the final volume to 250µl.
- 96-well. Transfer 0.1 - 0.5 µg of DNA directly into each well and adjust the final volume to 100µl.

Transfection of suspension cells (6-well)

GenePORTER 2 reagent works well for cells such as K562 and PC 12, which can grow in suspension. For 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 cell numbers indicated in Table 2 below in medium with or without serum, and transfer the complexes to the dish.

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

Table 2: Suggested Cell Numbers, Transfection Volumes and DNA Amounts

Tissue Culture Dish	Cell number/ well	Lipoplex volume/well	DNA amount/well	Total transfection volume/well
6-well	2,000,000 cells	250 µl	4 µg	1 ml
12-well	1,000,000 cells	125 µl	2 µg	500 µl
24-well	500,000 cells	62.5 µl	1 µg	250 µl
96-well	100,000 cells	31.25 µl	0.5 µg	100 µl

Notes:

[d] This step is important because some suspension cells have a tendency to clump, and the reagent has difficulty getting access to the cells in the center of these clumps. Gentle pipetting of cells disrupts these clumps and produces a true single-cell suspension, which will increase transfection efficiency.

[e] 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.

Suggested DNA optimization ranges for different tissue culture plates:

- 6-well. Transfer 3 - 5 μg of DNA directly into each well and adjust the final volume to 1ml.
- 12-well. Transfer 1 - 3 μg of DNA directly into each well and adjust the final volume to 500 μl.
- 24-well. Transfer 0.5 - 1.5 μg of DNA directly into each well and adjust the final volume to 250 μl.
- 96-well. Transfer 0.1 - 0.5μg of DNA directly into each well and adjust the final volume to 100 μl.

RELATED PRODUCTS

1. Reagent for enhanced transfection efficiency

BoosterExpress™ Reagent Kit is a set of chemical cocktails designed to significantly increase transfection efficiencies with any non-viral DNA transfection reagent. Simply add the appropriate Booster reagent to the culture medium 4 hours post transfection and get up to 12 times enhancement of gene expression levels. Each kit includes three Booster reagents and a comprehensive manual.

Product	**Catalog #**
BoosterExpress™ Reagent Kit	T20100B

2. Reporter vectors for transfection monitoring

gWIZ™/β-gal and gWIZ™/GFP are two reporter vectors that can be used to monitor transfection efficiency. They contain β-galactosidase (β-gal) or green fluorescence protein (GFP) gene, respectively. Both plasmids rely on the highly optimized CMV promoter/enhancer sequence for high-level expression of reporter molecules in mammalian cells.

Product	**Catalog #**
gWIZ/β-gal vector	P010200
gWIZ/GFP vector	P040400
β-galactosidase assay kits:	
Enhanced β-galactosidase assay kit (CPRG)	A10100K
β-galactosidase assay kit (ONPG)	A10200K
X-Gal staining assay kit	A10300K