A division of Gene Therapy Systems, Inc.

GenePORTER® Transfection Reagent QuikEase™ Single-Use Tubes

PRODUCT SUMMARY

Cat. No: **T201096**

Description: GenePORTER 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. The GenePORTER QuikEase Kit contains 96 single reaction tubes, which make transfection easier and more convenient. Each tube contains sufficient GenePORTER reagent for transfecting 1.25 µg or 2.0 µg of DNA. GenePORTER reagent is suitable for *in vitro* transfection as well as animal injection studies.

Components: 96 tubes of the dried GenePORTER lipid film

Storage: Store at 4 °C or 20 °C. Stable for at least 12 months when stored properly.

INTRODUCTION

GenePORTER® (Patent pending), which incorporates direct hydrophilic conjugation (DHC) technology, is the latest innovation in the DNA transfection field. GenePORTER reagent consistently delivers higher expression levels than other commercially available products in a wide variety of cell lines. This robust system is easy to use and does not require enhancers or special handling of cells, saving time, cost and money. GenePORTER reagent is especially recommended for delivering oligonucleotides and special cell lines such as Jurkat cells.

USAGE

Hydrate the GenePORTER lipid film with 250µl of DNA solution, pipette 5 times. Incubate at room temperature for 10 minutes, then transfer the complexes onto cells.

- For adherent cells, use 2µg DNA for each lipid tube.
- For suspension cells, use 1.25µg DNA for each lipid tube.

EXAMPLE PROTOCOLS

Transfection of adherent cells in a 6-well plate

1. The day before transfection, plate the cells so that they will be 60-70% confluent on the day of transfection.

2. Dilute 2 µg DNA in 250 µl of serum-free medium*.

3. Hydrate the GenePORTER reagent with the DNA solution. Pipette up and down 5 times. Incubation 10-20 minutes at room temperature.

4. Aspirate the culture medium from the cells. Add 0.75 ml of FCS-free medium to each well. Then carefully add the DNA-GenePORTER mixture onto the cells and incubate at 37°C for 4 hours.

5. 4 hours post transfection, add 1ml of medium containing 20% FCS. Continue to incubate overnight under 5-10% CO_2 at 37°C

6. 24 hours post transfection, add more fresh growth medium as needed. ** The assay can be done 24-72 hours after the start of transfection depending on the cell type and promoter activity. ***

Notes:

* PBS, TE and serum-free medium can be used. Avoid using the buffers with high phosphate content (>10mM NaPO4).

** For some cell types the old media can be replaced with fresh media at this step.

***The same protocol can be used to produce stably transduced cells except that 48 hours post transfection, cells are transferred to fresh medium containing the appropriate antibiotics for selection. It is important to wait at least 48 hours before exposing the transduced cells to selection media. For some cell types it may be necessary to wait as long as 4 to 5 days before applying the selection condition.

For different tissue culture plates:

- 96-well. Transfer 0.1-0.5 µg of DNA directly into each well (12.5-62.5 µl of DNA/GenePORTER complexes) and adjust the final volume to 100 µl.
- 24-well. Transfer 0.5-2 µg of DNA directly into each well (62.5-250 µl of DNA/GenePORTER complexes) and adjust the final volume to 250 µl.
- 12-well. Transfer 1-2 µg of DNA directly into each well (125-250 µl of DNA/GenePORTER complexes) and adjust the final volume to 500 µl to 1ml.

Transfection of suspension cells in a 6-well plate.

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

2. Dilute the 1.25 µg DNA with 250 µl serum-free medium.

3. Hydrate the GenePORTER reagent with the DNA solution, pipette up and down 5 times. Incubate at room temperature for 10-20 minutes.

4. While the DNA/GenePORTER complexes are forming, spin down the cells, resuspend them at 1,000,000 cells in 0.75 ml serum-free medium and transfer the cell suspension to the dish.

5. Add the DNA/GenePORTER complexes directly to the cells, and mix well by gently pipeting up and down 3-4 times *. Return cells to the incubator for 4 hours.

6. 4 hours post transfection, add 1ml of medium containing 20% serum**. Continue to incubate overnight at 37°C and 5-10% CO_2.

7. 24 hours post transfection, add more fresh growth medium as needed. Assay can be done 24-72 hours after the start of transfection depending on the cell type and promoter activity.

Notes:

* This step is important because suspension cells like Jurkat have a tendency to clump and the reagent has difficulty getting access to the cells in the center of these clumps. Gentle pipetting disrupts these clumps and produces a true single cell suspension that is easier to transfect.

** For some hematopoietic cell lines, such as Jurkat, PHA or PMA may be used at a final concentration of 1 μg/ml or 50 ng/ml, respectively, to enhance the levels of gene expression.

For different tissue culture plates:

- 96-well. Transfer 0.1-0.5 μg of DNA directly into each well (20-100 μl of DNA/GenePORTER complexes) and adjust the final volume to 100 μl. 100,000 cells /well.
- 24-well. Transfer 0.5-1.25 μg of DNA directly into each well (100-250 μl of DNA/GenePORTER complexes) and adjust the final volume to 250 μl. 500,000 cells / well.
- 12-well. Transfer 1.25 μg of DNA directly into each well (250 μl of DNA/GenePORTER complexes) and adjust the final volume to 500-1000 μl, 1,000,000 cells /well.

For some cells more DNA might be required. Consequently, dilute up to 2.5 μg of DNA in 250 μl of serum free medium and then hydrate the GenePORTER film.

RELATED PRODUCTS

1. Transfection in the presence of serum

GenePORTER 2 transfection reagent is an improved formulation that offers the highest transfection efficiency in most cell lines. In addition, it provides excellent results in the presence of serum. It is highly recommended for difficult-to-transfect cell lines.

Product	**Catalog #**
GenePORTER 2®Transfection Reagent	
QuikEase™ Kit (96 rxns.)	T202096
75 reactions (1.5 ml)	T202007
150 reactions (1.5 ml)	T202015
750 reactions (5 x 1.5ml)	T202075

2. 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

3. 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

Please contact us for a complete list of transfection, tranformation, gene expression and RNAi products.

Genlantis

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