GenePORTER®

Transfection Reagent

Genlantis
A division of Gene Therapy Systems, Inc.

Catalog #	Content	Amount
T201007 (75 Rxns.)	GenePORTER Transfection Reagent	1 vial (dry)
	Hydration Buffer	1 x 0.8 ml
T201015 (150 Rxns.)	GenePORTER Transfection Reagent	1 vial (dry)
	Hydration Buffer	1 x 1.6 ml
T201075 (750 Rxns.)	GenePORTER Transfection Reagent	5 vials (dry)
	Hydration Buffer	5 x 1.6 ml

Shipping	Shipped at room temperature.
Storage	Store at 4°C; stable for 1 year at 4°C.

RELATED PRODUCTS	Catalog Numbers
GenePORTER QuikEase™ Kit	T201096, 96 single-use tubes.
GenePORTER® 2 Transfection Reagent	T202007, 0.75 ml (75 reactions.)
	T202015, 1.5 ml (150 reactions.)
	T202075, 5 x 1.5 ml (750 reactions.)
GenePORTER® 2 QuikEase™ Kit	T202096, 96 single-use tubes.
GeneSilencer® siRNA Transfection Reagent	T500750, 0.75 ml (200 reactions.)
	T505750, 5 x 0.75 ml (1,000 reactions)
BioPORTER® Protein Delivery Reagent	BP502424, 24 single use tubes.
	BP509696, 96 single use tubes.
NeuroFect™ Transfection Reagent	T800075, 0.75 ml (75-300 reactions.)
	T800750, 5 x 0.75 ml (375-1500 rxns.)
Cytofectin™ Transfection Reagent (oligos)	T61001 (1 ml) T610005 (5 x 1 ml)

INTRODUCTION The GenePORTER Reagent is the original transfection lipid developed by Genlantis containing a unique formulation of the neutral lipid dioleoyl phosphatidylethanolamine (DOPE) and using the proprietary and innovative Direct Hydrophilic Conjugation (DHC) technology. The GenePORTER reagent is optimized for serum-free transfection conditions in a wide variety of commonly used cells. The GenePORTER reagent is especially effective in Jurkat cells and gives the highest transfection efficiencies relative to all other commercial reagents.

MATERIALS AND METHODS

A. GenePORTER® Hydration

Hydrate the GenePORTER lipid film at room temperature with 0.75 ml (for T201007) or 1.5 ml (for T201015 or T201075) of the Hydration Buffer. Vortex for 10 seconds at top speed. Store hydrated GenePORTER at 4°C and vortex briefly before each use.

B. Transfection of Adherent Cells

1. The day before transfection, plate the cells so that they will be 60-90% confluent on the day of transfection.

   NOTE: omitting antibiotics from the media during transfection may increase expression levels by a small amount.

2. Dilute the DNA with serum-free medium using half of the transfection volume. Use Table 1 below for suggested amounts:

Table 1: Amounts of Reagents for Transfection of Adherent Cells

Plate Type	DNA Amount (µg)	GenePORTER Volume (µl)	Transfection Volume (ml)
96 well	0.1-0.5	0.5-2.5	0.1
24 well	0.5-2.0	2.5-10	0.25
6 well	2.0-6.0	10.0-30.0	1.0
60 mm	6.0-8.0	30.0-40.0	2.0
100 mm	8.0-12.0	40.0-60.0	5.0

3. Add the diluted DNA to the diluted GenePORTER reagent. Mix rapidly and incubate at room temperature for 10-45 minutes.
4. Aspirate culture medium from cells; add the DNA/GenePORTER mixture gently to the cells. Incubate at 37°C in a 5-10% CO_2 incubator for 3-5 hrs.
5. Add one transfection volume of medium + 20% FCS. Continue to incubate overnight at 37°C in a 5-10% CO_2 incubator.
6. 24 hours post transfection, add fresh growth medium as needed.

   NOTE: For some cells, old media can be replaced with fresh media.

7. Perform cell assays 24-72 hours post transfection depending on cell type and promoter activity.

   NOTE: 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.

C. Transfection of Suspension Cells (e.g. Jurkat)

8. The day before transfection, plate the cells so that they will are in good condition the day of transfection.
9. Dilute the DNA with serum-free medium using ½ of the transfection volume. Use Table 2 below for suggested amounts:

Table 2: Amounts of Reagents for Transfection of Suspension Cells

Plate Type	Cells per Volume	DNA (µg)	GenePORTER Vol. (µl)	Transfection Volume (ml)
96 well	10^5/10 µl	0.1-0.5	0.8-1.6	0.1
24 well	$4x10^5$/40 µl	0.5-2.0	4.0-8.0	0.25
6 well	$2x10^6$/200 µl	2.0-6.0	16.0-32.0	1.0
60 mm	$4.5x10^6$/450 µl	6.0-8.0	48.0-64.0	2.0
100 mm	$1.2x10^7$/1.2 ml	8.0-12.0	64.0-96.0	5.0

NOTE: for 100 mm or larger dishes, it is recommended to prepare your DNA and GenePORTER reagent in 1 ml serum-free medium as for the 6-well plate, mix well, and further dilute to the desired volume by adding additional serum-free medium before transfection.

10. Same as Step 3.
11. While the DNA/GenePORTER complexes are forming, spin down the cells and resuspend them at 10 million cells/ml; transfer the appropriate number of cells to the dish (as shown in Table 2).

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12. Add the DNA/GenePORTER complexes to the cells and mix well by gently pipetting up and down 3 to 4 times.

    NOTE: this step is important because some suspension cells have a tendency to clump, which reduces transfection efficiency.

13. Return the cells to the incubator for 3-5 hours.
14. 3-5 hours post-transfection, add one transfection volume of medium + 20% FCS.

    NOTE: 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.
15. Continue to incubate overnight at 37°C and 5-10% CO_2.
16. 24 hours post transfection, add fresh growth medium as needed.
17. Perform cell assays 24-72 hours post transfection depending on cell type and promoter activity.

D. DETECTION OF EXPRESSED REPORTER GENES

β-Galactosidase

The following protocol[1] is provided for your convenience (alternatively, you could use one of the β-galactosidase assay kits offered by Genlantis Catalog Numbers: A10100K, A10200K, and A10300K):

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 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.* (1994) Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. *J Biol Chem* **269**: 2550-2561.
2. Felgner, PL. *et al.* (1987) Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. *Proc Natl Acad Sci USA* **84**: 7413-7417.
3. Gussoni, E. *et al.* (1996) A method to codetect introduced genes and their products in gene therapy protocols. *Nature Biotechnology* **14**: 1012-1015.

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