GenePORTER® Gold

Transfection Reagent

Genlantis

Catalog #	Contents	Quantity
T204001S (10 rxns.)	GenePORTER® Gold Reagent, Trial Size	1 x 0.1 ml
	GP Gold Diluent	1 x 0.5 ml
	GP Gold Enhancer	1 x 0.1 ml
T204015 (400 rxns.)	GenePORTER® Gold Reagent	1 x 1.5 ml
	GP Gold Diluent	1 x 10.0 ml
	GP Gold Enhancer	1 x 1.0 ml
T204030 (800 rxns.)	GenePORTER® Gold Reagent	2 x 1.5 ml
	GP Gold Diluent	2 x 10.0 ml
	GP Gold Enhancer	2 x 1.0 ml
T204115 (4,000 rxns.)	GenePORTER® Gold Reagent	1 x 15 ml
	GP Gold Diluent	1 x 100.0 ml
	GP Gold Enhancer	1 x 10.0 ml

Related Products	Catalog #
GenePORTER® 2 Transfection Reagent, 75 reactions	T202007
GenePORTER® 2 Transfection Reagent, 150 reactions	T202015
GenePORTER® 2 Transfection Reagent, 750 reactions	T202075
BoosterExpress™ Reagents, 3 x 1.5 ml	T20100B
GeneSilencer® siRNA Transfection Reagent, 200 reactions	T500750
GeneSilencer® siRNA Transfection Reagent, 1000 reactions	T505750
phCMV1 Expression Vector Kit (native expression)	P003100
phCMV1 Expression Vector Kit (N-terminal fusion)	P003200
phCMV1 Expression Vector Kit (C-terminal fusion)	P003300
Enhanced β-galactosidase Assay Kit (CPRG)	A10100K
β -galactosidase Assay Kit (ONPG)	A10200K
X-gal Staining Assay Kit	A10300K
NeuroFect™ Transfection Reagent, 75-300 reactions	T800075
NeuroFect™ Transfection Reagent, 375-1500 reactions	T800750

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

Introduction: The GenePORTER® Gold Transfection Reagent is our newest, most effective and reproducible lipid based gene delivery formulation for a wide variety of cell lines. It utilizes our Advanced Carrier Enhancement (ACE) technology that maximizes transfection efficiency and transgene expression levels with minimal cytotoxicity. The transfection efficiency levels achieved with GenePORTER Gold match or surpass other commercially available reagents. The GenePORTER Gold kit features an easy to use protocol and enough reagent for 400 x 1ug transfections to ensure that you get maximum transfection efficiency and value per reaction in the broadest spectrum of cell lines.

METHODS AND PROCEDURES

For optimal DNA transfection conditions, we recommend using cells that are 75% to 85% confluent on transfection day. Typically, for experiments in 12-well plates, 100,000 (e.g. CHO-K1) to 200,000 (e.g. HEK 293) cells are seeded per well in 1 ml of cell growth medium 24 hours prior to transfection. For other plate formats refer to Table 1 for recommended cell seeding densities and corresponding media volumes.

NOTE: Optimal cell density is cell line specific and should be optimized.

Table 1: Number of Cells and Media Volumes Recommended per Plate Type

Plate Type	Recommended Number of Cells per Well/Plate	Medium Volume per Well/Plate (ml)
96-well	8,000-12,000	0.1
24-well	50,000-100,000	0.5
12-well	100,000-200,000	1.0
6-well	250,000-450,000	2.0
60 mm	275,000-825,000	4.0
100 mm	2 x 10^6-6 x 10^6	7.0

Next, use Table 2 below for amounts needed in the General Protocol Section below.

Table 2: Recommended Amounts per Plate Type

Plate Type	DNA Range (µg)	GP Gold Diluent (µl)	GP Gold Enhancer	GP Gold Reagent (µl)	Transfection Volume (µl)
96-well	0.1-0.5	6.25	0.5-1.µl	0.35-1.75	15.0
24-well	0.5-1.0	12.5	1-5 µl	1.75-3.5	75.0
12-well	1.0-2.0	25.0	5-10 µl	3.5-7.0	150.0
6-well	2.0-6.0	50.0	10-30 µl	14.0-21.0	300.0
60 mm	6.0-8.0	100.0	30-40 µl	21.0-28.0	600.0
10 cm	9.0-12.0	200.0	45-60 µl	31.5-42.0	1,000.0

NOTE: The optimal ratio of reagents for 1 µg of plasmid DNA is 25 µl GP Gold Diluent + 5.0 µl GP Gold Enhancer + 3.5 µl GP Gold transfection reagent in a total transfection volume of 150 µl.

General Protocol

For optimal DNA transfection we recommend passaging the cells in antibiotic–free medium at least 24 hours before plating cells. Perform the GenePORTER Gold transfection reactions in serum-free medium (e.g. OptiMEM from Life Technologies, Inc.) for a minimum of 4 hours. Remove the transfection reaction and add antibiotic-free complete growth medium. Incubate for an additional 24-48 hours followed by your downstream assays.

1. Prepare the DNA Mix by adding the following components in this order:

   GP Gold Diluent, **then** GP Gold Enhancer, **then** plasmid DNA. Use the amounts recommended in Table 2 above.
2. Vortex and incubate at room temperature for 5 minutes.
3. In the meantime, use Table 2 to calculate the amount of serum-free media needed to dilute the GenePORTER Gold Reagent; use the following formula:

   Serum-free medium volume (µl) = Transfection Volume (µl) – GP Gold Diluent (µl) – GP Gold Enhancer (µl) – DNA volume (µl) – GP Gold Reagent (µl).

   EXAMPLE: for a 12-well plate and a 1 µg/µl DNA solution, the serum-free medium volume needed to dilute the GenePORTER Gold Reagent is:

Recommended transfection rxn. volume:	150.0 µl
GP Gold Diluent:	-25.0 µl
GP Gold Enhancer:	-5.0 µl
Plasmid DNA:	-1.0 µl
GP Gold Reagent:	-3.5 µl
Serum-free medium (vol) to dilute GP Gold	115.5 µl

4. Add GenePORTER Gold Reagent to Serum-free medium calculated in step 3. Incubate 5 minutes @ room temperature.
5. In this order, add the diluted GenePORTER Gold Reagent from Step 4 to the DNA Mix in Step 2 above. This is the **Transfection Mix**.
6. Vortex briefly and incubate for 5 minutes at room temperature.

   IMPORTANT: do not incubate the Transfection Mix for more than 30 minutes.
7. Meanwhile, remove complete medium from overnight plated cells and replace with an equal volume of serum-free medium.
8. Add the Transfection Mix from Step 6 to the cells in step 7.
9. Incubate the transfection reaction for 4 hours.
10. Remove the entire transfection reaction volume and replace it with complete medium using the volumes recommended in Table 1 above.
11. Incubate the cells for 24-72 hours then perform your downstream assay or experiment. Typically, we recommend a minimum of 48 hours for both optimal efficiency and transgene expression levels.

Optimization Guidelines for Difficult to Transfect Cells

The GenePORTER Gold Reagent consistently delivers high transfection efficiencies in a wide range of cell types, however optimization for your target molecule may be desired. The four critical optimization variables are:

1) Quantity of DNA;

2) Ratio of GP Gold Diluent to DNA;

3) Ratio of GP Gold Enhancer to DNA and

4) Ratio of GenePORTER Gold Reagent to DNA.

To optimize: 1) Vary the quantity of DNA +/- 50% from the recommended amount in Table 2; 2) Vary the GP Gold Diluent volume using 17.0, 25.0, 50.0, 100.0 and 150.0 µl per 1 µg of DNA; 3) Maintain a ratio of 1 ug of DNA to 5 µl GP Gold Enhancer; 4) Vary the GenePORTER Reagent volume using 1.75 µl, 3.5 µl and 7.0 µl per 1 µg of DNA. Use a low DNA quantity to optimize the ratios. Following this process, cell number can also be optimized. For an example optimization set-up, please visit the GenePORTER Gold page at www.genlantis.com.