GenePORTER® 3000

Transfection Reagent

Catalog #	Contents	Quantity
T203001S (10 reactions)	GenePORTER® 3000 Reagent, Trial Size	0.1 ml
	GP3K Diluent	500.0 µl
T203007 (107 reactions)	GenePORTER® 3000 Reagent	0.75 ml
	GP3K Diluent	6.0 ml
T203015 (214 reactions)	GenePORTER® 3000 Reagent	1.5 ml
	GP3K Diluent	2 x 6.0 ml
T203115 (2,140 reactions)	GenePORTER® 3000 Reagent	2 x 7.5 ml
	GP3K Diluent	120.0 ml

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

Related Products	Catalog # (Size)
Detachin™ Cell Detachment Solution	T100100 (100 ml); T100106 (6 x 50 ml); T100110 (10 x 100 ml).
GenePORTER® Gold Transfection Reagent	T204015 (1.5 ml); T204030 (2 x 1.5 ml); T204115 (15 ml).
GeneSilencer® siRNA Transfection Reagent	T500750 (200 reactions); T505750 (1,000 reactions).
BioPORTER® Protein Delivery Reagent	BP502401 (24 reactions); BP509604 (96 reactions).
BioPORTER® Protein Delivery Reagent, QuikEase kit	BP502424 (24 reactions); BP509696 (96 reactions).

Introduction: The GenePORTER 3000® Transfection Reagent (GP3K) is a proprietary cationic lipid formulation that utilizes Advanced Carrier Enhancement (ACE), a unique targeting technology that maximizes plasmid delivery to the cell nucleus while minimizing cytotoxicity. The transgene expression levels achieved with GP3K frequently surpass those achieved with competing reagents, especially in hard to transfect cell lines like RAW 264.7. The GP3K Reagent also allows for a rapid same-day transfection protocol saving you substantial time for downstream applications. Effective in a broad range of cell types, GP3K provides superior delivery efficiency, high transgene expression levels, and a flexible protocol, making it ideal for most delivery and protein expression experiments.

METHODS AND PROCEDURES

A. General Notes

1. GenePORTER 3000 is optimized for both standard transfection in overnight plated cells and same-day transfection using freshly plated cells. Depending on your experimental goals, we recommend a preliminary experiment comparing both seeding methods to determine optimal efficiency and transgene expression levels. Typically, we achieve the highest and most reproducible transfection efficiencies and transgene expression levels in overnight plated cells in serum-free medium.
2. When performing same day transfection with fresh plated cells, experimental preparation will take about 30-45 minutes. This is followed by a 4-hour incubation of the GenePORTER 3000/DNA complexes with cells in serum-free medium.
3. For most cell types, we recommend a ratio of 7 µl of GenePORTER 3000 Reagent per 1 µg of DNA pre-diluted in 50 µl of GP3K Diluent.
4. The serum free protocol requires preparation of complete medium containing 20% serum for step D4d.
5. We recommend removing antibiotic from the cultured cells at least 24 hours prior to transfection.
6. Your DNA can be suspended in TE buffer or purified water. A DNA concentration of at least 0.1 mg/ml works well for most reactions.
7. For RAW 264.7 transfection follow the protocol in Section F below.

B. Preparation of Adherent Cells

Option 1: Overnight Plating

1. Maintain low passage number, healthy, log phase cells in culture without antibiotics for at least 24 hours pre-transfection.
2. On the day before transfection, seed plates in complete medium according to the recommended per well density in Table 1.

   NOTE: For optimal DNA transfection, we recommend using cells that are 75% to 85% confluent on the day of transfection. Typically, for experiments in 12-well plates, we seed 100,000 (e.g. CHO-K1) to 200,000 (e.g. RAW 264.7) cells 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 plating densities and media volumes. Optimal cell density is cell line specific and should be determined empirically.

Table 1: Recommended Overnight Cell Plating Densities and Media Volumes.

Plate Type	Cell Density per Well	Approx. Media Volume (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

Option 2: Same Day Plating

Maintain low passage number, healthy, log phase cells in culture without antibiotics for at least 24 hours pre-transfection.

3. On the day of transfection, prepare cells for plating using Detachin™ Cell Detachment Solution (see Related Products on Page 8) as indicated below to maximize cell viability.
   NOTE: Trypsin/EDTA may be used in place of Detachin, however cell viability may decrease.
4. Remove medium from cells and wash with 1X DPBS.
5. Add 1-2 ml of room temperature Detachin Solution per 75 cm2 of substrate. Rock container back and forth to cover surface area with Detachin.
6. Incubate 3-5 minutes (37C + 5% CO2) until the cells detach.
   NOTE: For some cell lines like RAW 264.7, use a cell scraper in addition to Detachin treatment.
7. Add 5 volumes of complete medium and mix by pipetting.
8. Transfer suspended cells to a conical tube and centrifuge at 1,000 x g for 5 minutes at room temperature.
9. Resuspend pellet in complete medium, and determine cell count and viability.
10. For optimal performance, plate at a high cell density in complete medium as indicated in Table 2:

Table 2: Recommended Adherent Cells Plating Densities and Media Volumes.

Plate Type	Cell Density per Well	Approximate Volume (ml)
96-well	5.0 x 10^4	0.1
48-well	1.5 x 10^5	0.2
24-well	3.0 x 10^5	0.5
6-well	1.5 x 10^6	2.0
60 mm	3.2 x 10^6	5.0
100 mm	8.25 x 10^6	12.0

11. Incubate freshly plated cells for a minimum of 30 minutes (37°C + 5% CO_2) prior to transfection.

C. Transfection Protocol for Adherent Cells

1. Dilute the DNA in GP3K Diluent as shown in Table 3 below. Mix well by briefly vortexing and incubate for 5 minutes at room temperature.

Table 3: DNA Dilution in GP3K Diluent.

Plate Type	GP3K Diluent (µl)	DNA Amount (µg)
96-well	12.5	0.25
48-well	25.0	0.5
24-well	50.0	1.0
6-well	200.0	4.0
60 mm	350.0	7.0
100 mm	500.0	10.0

NOTE: See Section G for optimization guidelines if needed.

2. Prepare the GP3K Reagent Dilution by diluting GP3K Reagent in serum-free medium (e.g. OptiMEM, Life Technologies, Inc.) in a separate tube as shown in Table 4 below. Incubate for 5 minutes at room temperature.

Table 4: GP3K Reagent Dilution in Serum Free Medium.

Plate Type	Serum-Free Media (µl)	GP3K Reagent (µl)
96-well	7.0	1.75
48-well	14.0	3.5
24-well	28.0	7.0
6-well	112.0	28.0
60 mm	196.0	49.0
100 mm	280.0	70.0

NOTE: See Section G for optimization guidelines if needed.

3. To form the Transfection Mix (lipoplexes), combine the GP3K Reagent Dilution from step D2 to the DNA GP3K Diluent Mix from step D1 (in that order). Vortex briefly and incubate 5 minutes (no longer than 30 minutes) at room temperature.
4. For Serum-free Medium Transfections

   NOTE: For highest efficiency, we recommend transfecting all cell lines in serum-free medium for 4 hours. However, some cell lines can be transfected with high efficiency in the presence of serum. Refer to Section H, Table 8 for optimized transfection conditions for some commonly used cell lines.

   a. Remove the complete medium from the cells plated in step C11. Immediately replace with an equal volume of serum-free medium.
   b. Add Transfection Mix from Step D3 directly to cells in serum free medium in step D4a.
   c. Swirl gently to mix; incubate (37°C+5% CO2) for exactly 4 hours.
   d. Add an equal volume of complete medium containing 20% serum for a final well concentration of 10% serum.
   e. Incubate (37°C + 5% CO2) and assay cells after 48-72 hours.
5. For Complete Medium Transfections
   a. Add the Transfection Mix from step D3 to the cells plated in step C11.
   b. Incubate (37°C + 5% CO2) and assay cells after 48-72 hours.

D. Transfection Protocol for Adherent Cells

Prepare suspension cells on the day of transfection. Centrifuge at 1,000 x g for 5 minutes at room temp to separate out your cells. Transfer cells to serum-free medium at the recommended cell densities in **Table 5** below. Proceed with transfection following step D4.

Table 5: Recommended Suspension Cells Plating Densities.

Plate Type	Cell Density per Well
96-well	9.0×10^4
48-well	3.0×10^5
24-well	6.0×10^5
6-well	3.0×10^6
60 mm	6.0×10^6
100 mm	1.65×10^7

E. Transfection Protocol for RAW 264.7 Cells

For RAW 264.7 cells use the same protocol as the Transfection of Adherent Cells in Section D above, but use **Table 6** below for recommended DNA dilution in GP3K Diluent, and **Table 7** below for GP3K Reagent Dilution. For optimization guidelines, see Section G below if needed

Table 6: DNA Dilution in GP3K Diluent for RAW 264.7 Cells.

Plate Type	GP3K Diluent (µl)	DNA Amount (µg)
96-well	37.5	0.25
48-well	75.0	0.5
24-well	150.0	1.0
6-well	600.0	4.0
60 mm	1050.0	7.0
100 mm	1500.0	10.0

Table 7: GP3K Reagent Dilution Preparation in Serum Free Medium.

Plate Type	Serum-Free Media (µl)	GP3K Reagent (µl)
96-well	14.0	3.5
48-well	28.0	7.0
24-well	56.0	14.0
6-well	224.0	56.0
60 mm	392.0	98.0
100 mm	560.0	140.0

F.

Optimization Guidelines for Difficult to Transfect Cells

GenePORTER 3000 reagent consistently delivers high transfection efficiencies in a wide range of cell types; however, optimization for your target molecule may be desired. The three critical optimization variables are:

a) quantity of DNA;

b) ratio of GP3K Diluent to DNA;

c) ratio of GP3K Reagent to DNA.

To optimize:

1) vary the DNA quantity +/- 50% of the recommended amount in Tables 3 and 6;

2) vary the GP3K diluent volume using 17.0, 25.0, 50.0, 100.0 and 150.0 µl per 1 µg of DNA;

3) vary the GP3K Reagent volume using 4.7, 5.25, 7.0, 10.0 and 14.0µl 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 Transfection Optimization Table link on the GenePORTER 3000 Transfection Reagent page at **www.genlantis.com.**

G. Optimized Transfection Conditions for Commonly Transfected Cells

We have found the following conditions to be optimal for the cell lines listed below based on transfection of a 5 kb GFP plasmid in 24-well plates.

Table 8: Optimized Transfection Conditions for Commonly Transfected Cells

Cell Line	Cell Plating	Serum or Serum-Free	GP3K Diluent (µl)	GP3K Reagent (µl)	DNA (µg)
COS-7	O/N	SF	50	7	1
CHO-K1	O/N	SF	50	3.5-7	1
Jurkat*	SD	SF	N/A	3.5-7	1
HeLa	Both	Both	16.7-50	2.3-7	1
HEK-293	O/N	Both	50	7	1
HepG2	Both	SF	50	7	1
MCF-7	Both	SF	50	7	1
NIH-3T3	O/N	SF	50	7	1
RAW 264.7	Both	SF	50-150	7-21	1-3

Table Key: **O/N**=Overnight / **SF**=Serum-free / **SD**=Same-day
*Jurkat cells do not use GP3K Diluent. Dilute DNA in serum-free medium (same volume as that indicated for GP3K Diluent).

AMS BIOTECHNOLOGY (EUROPE) LTD
UK & REST OF THE WORLD
184 Park Drive, Milton Park
Abingdon, OX14 4SE
T: +44 (0) 1235 828 200
F: +44 (0) 1235 820 482

AMSBIO LLC
USA & CANADA
1035 Cambridge Street,
Cambridge, MA 02141
T: +1 (617) 945-5033 or
+1 (800) 987-0985
F: +1 (617) 945-8218

AMS BIOTECHNOLOGY (EUROPE) LTD
SWITZERLAND
Via Lisano 3, (Cp.683)
Ch-6900 Messagno
T: +41 (0) 91 604 55 22
F: +41 (0) 91 605 17 85

AMSBIO EUROPE BV
EU
Berenkoog 41,
1822 BH Alkmaar,
Netherlands
T: +31 (0) 72 8080244
F: +31 (0) 72 8080142