

GenePORTER[®] 3000



A division of Gene Therapy Systems, Inc.

Transfection Reagent

Catalog #	Contents	Quantity
T203007 (75 reactions)	GenePORTER [®] 3000 Lipid	0.75 ml
	GP3K-Diluent	4 ml
T203015 (150 reactions)	GenePORTER [®] 3000 Lipid	1.5 ml
	GP3K-Diluent	8 ml
T203115 (1,500 reactions)	GenePORTER [®] 3000 Lipid	2 x 7.5 ml
	GP3K-Diluent	80 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 [®] 2 Transfection Reagent	T202007 (0.75 ml); T202015 (1.5 ml); T202075 (5 x 1.5 ml)
GenePORTER [®] Transfection Reagent	T201007 (0.75 ml); T201015 (1.5 ml); T201075 (5 x 1.5 ml)
GeneSilencer [®] siRNA Transfection Reagent	T500750 (200 reactions); T505750 (1,000 reactions)
NeuroFect™ Transfection Reagent	T800075 (75 reactions); T800750 (1,500 reactions)
BioPORTER [®] Protein Delivery Reagent	BP502401 (24 reactions); BP509604 (96 reactions)
BioPORTER [®] Protein Delivery Reagent, QuikEase kit	BP502424 (24 reactions); BP509696 (96 reactions)
Cytofectin™ Transfection Reagent, 1 ml	T610001 (1 ml); T610005 (5 x 1ml)

Introduction: The GenePORTER 3000[®] Transfection Reagent (GP3K) is a proprietary cationic lipid formulation that utilizes Advanced Carrier Enhancement (ACE) technology to maximize target molecule delivery with minimal cytotoxicity. The transgene expression levels achieved with GP3K far surpass those achieved with competing reagents, especially in hard to transfect cell lines like RAW 264.7. The GP3K reagent allows for a rapid, same-day transfection protocol in a broad range of cell lines. The superior delivery efficiency and transgene expression levels, combined with a simple and rapid protocol make GP3K reagent ideal for all of your delivery and protein expression experiments.

METHODS AND PROCEDURES

A. General Notes

- GenePORTER 3000 is optimized for same-day transfection using freshly plated cells. Cell preparation and assembly of the transfection reactions will take approximately 30-45 minutes. This may be followed by a 4-hour incubation in serum-free medium.
- For most cell types, we recommend to use 7 µl of GPK reagent per 1 µg of DNA pre-diluted in 50 µl of GP3K Diluent.
- The serum free protocol requires preparation of complete medium containing 20% serum for **step 21d**.
- Do not remove transfection complexes after adding them to your cells in order to avoid reducing efficiency and expression levels.
- We recommend removing antibiotic from the cultured cells at least 24 hours prior to transfection.
- 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 rxns.
- For RAW 264.7 transfection** follow the protocol in **Section D** below.

B. Transfection of Adherent Cells

- Maintain low passage number, healthy log phase cells in culture (without antibiotics 24 hours pre-transfection) so that they are 60-80% confluent on day of transfection.
- On the day of transfection, prepare cells for plating using Detachin™ Cell Detachment Solution (available separately; see Related Products above) to maximize cell viability.

NOTE: Trypsin/EDTA may be used in place of Detachin, however cell viability may go down.

- Remove medium from cells and wash with 1X DPBS.
- Add 1-2 ml of room temperature Detachin Solution per 75 cm² of substrate.
- Incubate 3-5 minutes (37°C + 5% CO₂) until the cells detach.
NOTE: For some cell lines like RAW 264.7, you may need to use a cell scraper in addition to Detachin treatment.
- Add 5 volumes of complete medium and mix by pipetting.
- Transfer suspended cells to a conical tube and centrifuge at 1,000 x g for 5 minutes at room temperature.
- Resuspend pellet in complete medium, and determine cell count and viability.
- For optimal performance, plate at a high cell density in complete medium as indicated in Table 1:

Table 1: Recommended Adherent Cells Plating Densities

Tissue Culture 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	0.20
24-well	3.0 x 10 ⁵	0.50
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

- Incubate cells for a minimum of 30 minutes (37°C + 5% CO₂).
- Dilute the **DNA in GP3K-Diluent** as shown in Table 2 below. Mix well by briefly vortexing and incubate for 5 minutes at room temperature.

Table 2: 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 E for optimization guidelines

- Prepare the **GP3K Reagent Dilution** by diluting GP3K in serum-free medium (e.g. OptiMEM, Invitrogen Cat. No. 110528) in a separate tube as shown in Table 3 below. Incubate for 5 minutes at room temperature.

Table 3: 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 E for optimization guidelines

- To form the **Transfection Mix** (lipoplexes), combine the DNA GP3K-Diluent Mix in **Table 2** to the GP3K Reagent Dilution in **Table 3** (in that order). Vortex briefly and incubate 5 minutes (no longer than 30 minutes) at room temperature.
- For Serum Free Medium Transfections**
 - Remove the complete medium from the cells plated in **step 17**. Immediately replace with an equal volume of serum-free medium.
 - Add Transfection Mix from **step 20** directly to cells in serum free medium in **step 21 a**.
 - Swirl gently to mix; incubate (37°C+5% CO₂) for exactly 4 hours.
 - Add an equal volume of complete medium containing 20% serum for a final well concentration of 10% serum.
 - Incubate (37°C + 5% CO₂) and assay cells after 48-72 hours.
- For Complete Medium Transfections**
 - Add the Transfection Mix from **step 20** to the cells plated in **step 17**.
 - Swirl gently to mix; incubate (37°C+5% CO₂).
 - Assay cells after 48-72 hours.

C. For Suspension Cells:

For suspension cells, begin at Step B8 above. Centrifuge at 1,000 x g for 5 minutes at room temp to separate out your cells. Transfer cells to serum-free or serum-containing medium at the cell densities in **Table 4** below. Proceed with transfection following Step 21 or 22.

Table 4: Recommended Suspension Cells Plating Densities

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

D. For RAW 264.7 Cells

For RAW 264.7 cells use the same protocol as the Transfection of Adherent Cells in **Section B** above, but use **Table 5** below for recommended DNA dilution in GP3K, and **Table 6** below for GP3K Reagent Dilution. For optimization guidelines, see Section E.

Table 5: 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 6: 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

E. 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: **1)** Quantity of DNA. **2)** Ratio of GP3K Diluent to DNA and **3)** Ratio of GP3K reagent to DNA. To optimize: **1)** Vary the DNA quantity +/- 50% of the recommended amount in Tables 2 and 5, **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 14.0 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 <http://tinyurl.com/dcndlv> on the Internet.

LIMITED LICENSE: The purchase price paid for the GenePORTER® 3000 Transfection Reagent Kits (hereto "GP3K") grants end users a non-transferable, non-exclusive license to use the kits and/or their components for **internal research use only** as described in this manual; in particular, research use only excludes and without limitation, resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of Genlantis, a division of Gene Therapy Systems, Inc. (GTS) -- separate licenses are available for non-research use or applications. GP3K and/or its components are not to be used for human diagnostic or included/used in any drug intended for human use. Care and attention should be exercised in handling the kit components by following appropriate research laboratory practices. Purchasers may refuse this license by returning the enclosed materials unused. By keeping or using the enclosed materials, you agree to be bound by the terms of this license. The laws of the State of California shall govern the interpretation and enforcement of the terms of this License.