

**Frequently Asked Questions (FAQ's):
GenePORTER™/GenePORTER™ 2 Transfection Reagents**

1. **What are the GenePORTER Transfection Reagents?**
2. **What is the difference between GenePORTER and GenePORTER 2?**
3. **How do the GenePORTER Transfection Reagents work?**
4. **Are the GenePORTER reagents suitable for particular cell types?**
5. **For GenePORTER 2, what is the difference between the original DNA Diluent and the DNA Diluent B?**
6. **How is one reaction (transfection) defined for the GenePORTER reagents?**
7. **How soon after the onset of transfection can I assay for gene expression?**
8. **How do I optimize my transfection procedure using the GenePORTER reagents?**
9. **Can the GenePORTER reagents be used for delivering oligonucleotides?**
10. **Where can I find published research that cites the use of the GenePORTER reagents?**
11. **How stable are the GenePORTER reagents before and after hydration?**
12. **How cytotoxic are the GenePORTER reagents?**
13. **Why isn't there anything visible in the vial containing the GenePORTER reagents?**
14. **Can the GenePORTER reagents be used for in-vivo experiments?**
15. **Are the GenePORTER reagents suitable for stable transfection?**
16. **Can the GenePORTER reagents be used for co-transfection (transfection of more than one plasmid)?**
17. **Can the GenePORTER reagents be used on quiescent (non-dividing) cells?**
18. **Is there any "auto-fluorescence" associated with the GenePORTER reagents?**
19. **What are the precipitates that settle on top of the cells after adding the DNA/GenePORTER Lipoplexes, which are visible under light microscopy?**
20. **Can the GenePORTER/DNA complexes be saved for another day?**
21. **What is the difference between the BoosterEXPRESS™ reagents and the DNA Diluents contained in the GenePORTER 2 kit? Are they comparable?**
22. **Can I use BoosterEXPRESS with my own transfection reagent?**
23. **In what buffer and at what concentration should my DNA be suspended prior to diluting it with serum-free media (or DNA diluents for GenePORTER 2)?**

1. What are the GenePORTER Transfection Reagents?

The GenePORTER and GenePORTER 2 Transfection Reagents are polyvalent, cationic lipids that allow high transfection efficiencies to be achieved in a broad range of cell types. Additionally, these reagents provide low cytotoxicity, exceptional stability, and ease of use.

2. What is the difference between GenePORTER and GenePORTER 2?

The GenePORTER Transfection Reagent allows efficient serum-free transfection of a broad spectrum of commonly transfected cells, such as HEK293, COS-1, COS-7, B16-F0, and Jurkat. The GenePORTER Reagent is easy to use, minimally toxic, and ideal for both adherent and suspension cells. The GenePORTER 2 Reagent is especially effective in difficult-to-transfect cells, such as PC-12, macrophages, and primary cells. The GenePORTER 2 Reagent provides high transfection efficiency, low cytotoxicity, and exceptional stability. Additionally, because of its minimized interaction with serum components, the GenePORTER 2 reagent is ideal for transfection in serum containing media.

3. How do the GenePORTER Transfection Reagents work?

The positively charged primary amino functional groups in the polar region of the GenePORTER transfection reagents interact with the negatively charged phosphate backbone of the plasmid



DNA, RNA or oligonucleotides electrostatically. The newly formed DNA/GenePORTER complexes have a net positive charge (when formulated under the recommended conditions), and will bind to the negatively charged plasma membranes of the cells. The complexes are subsequently taken inside the cells via endocytosis.

4. Are the GenePORTER reagents suitable for particular cell types?

The GenePORTER and the GenePORTER 2 transfection reagents are suitable for working with both adherent (e.g. COS, NIH-3T3, MCF-7) and suspension cultured (e.g. Jurkat, MDCK, K-562) cells, and with both primary and immortalized cells. From continuously surveying our customers, we have identified over 100 cell types that have been successfully transfected using GenePORTER reagents. Please visit our website at www.genetherapysystems.com to see a list of cells successfully transfected using the GenePORTER transfection reagents.

5. For GenePORTER 2, what is the difference between the original DNA Diluent and the DNA Diluent B?

The GenePORTER 2 DNA Diluent and New DNA Diluent B each contain different proprietary components that enhance transfection efficiency in different cell types. Both DNA diluents are supplied with the GenePORTER 2 lipid to allow customers to achieve the highest transfection efficiencies in the broadest range of cell. The GenePORTER 2 protocol contains a table for which DNA diluent to use with 16 commonly transfected cells. If the cells under study are not among those listed, we recommend starting with the new DNA Diluent B.

6. How is one transfection reaction defined for the GenePORTER reagents?

One transfection reaction for the GenePORTER reagents is defined as delivery of 2 ug of DNA in one well of a 6-well plate or in a 35mm dish of cells. Please note that GenePORTER reagent transfection conditions are not based on the surface area or the volume of the culture dish, but rather the number of cells plated. Please refer to the protocols accompanying the product.

7. How soon after the onset of transfection can I assay for gene expression?

Typically, assaying for reporter gene activity may be performed 24-72 hours after the onset of transfection. However, when using the GenePORTER reagents with very slow dividing cells, it is recommended to wait for at least one cell division before assaying for the transgene product.

8. How do I optimize my transfection procedure using the GenePORTER reagents?

First, it is important to be sure that the vector carrying the gene of interest has been optimized for high expression levels (e.g., pCMV and gWIZ vectors). If the vector has not been optimized, the level of gene expression could be minimal or none.

Second, transfection efficiency may potentially be increased by adjusting the quantity of DNA and the ratio of GenePORTER reagent to DNA:

For GenePORTER Transfection Reagent:

- a. Adherent Cells. First determine the best ratio of GenePORTER /DNA by using 3 – 9 ul of GenePORTER for each 1 ug of DNA. Use a low quantity of DNA to optimize this ratio. Once the optimum ratio is determined, vary the DNA quantity over the suggested range. At this point, cell number can also be optimized.
- b. Suspension Cells. Use the same optimization procedure as for adherent cells, except that the GenePORTER/DNA ratio is higher. Use 6-14 ul of GenePORTER for each 1 ug of DNA.

For GenePORTER 2 Transfection Reagent:

- a. Adherent Cells. First maintain a fixed ratio GenePORTER 2/DNA and vary the quantity of DNA over the suggested range. Then, if necessary, optimize the ratio of GenePORTER / DNA by using 3 to 6 ul of GenePORTER 2 for each 1 ug of DNA. Following this process, cell numbers can also be optimized.



b. Suspension Cells. Use the same optimization procedure as for adherent cells.

9. Can the GenePORTER reagents be used for delivering oligonucleotides?

The GenePORTER reagent is recommended for delivery of oligonucleotides (though the GenePORTER 2 reagent has also been used for this application). The GenePORTER reagents have been shown to deliver phosphothioate oligonucleotides into cells with ease. Also, the GenePORTER reagents have been used to deliver 20-mer to 80-mer oligos into a variety of mammalian cell lines. (Note: It is not recommended to use the GenePORTER reagents with neutral or positively charged DNA analogues.)

10. Where can I find published research that cites the use of the GenePORTER reagents?

Please visit our website at www.genetherapysystems.com to see a list of published references that cite the use of the GenePORTER transfection reagents.

11. How stable are the GenePORTER reagents before and after hydration?

For maximum stability, store all components of the GenePORTER Reagent kit at 4°C upon receipt. If stored properly, the dried GenePORTER™ 2 reagent is stable for 12 months, and the hydrated GenePORTER™ 2 reagent is stable for 6 months. The Hydration Buffer and DNA Diluent are stable for 12 months.

12. How cytotoxic are the GenePORTER reagents?

GenePORTER and GenePORTER 2 have minimal cytotoxicity under normal working conditions. In fact, many researchers have chosen to use the GenePORTER reagents specifically because of the minimized cytotoxicity.

13. Why isn't there anything visible in the vial containing the dry GenePORTER reagent?

The GenePORTER reagents are provided as a dry lipid film to increase long-term stability. The lipid film is essentially invisible to the naked eye, but it is there. Just hydrate the product as instructed in the protocol. (NOTE: In the event that the hydration buffer is accidentally spilled or missing, sterile tissue culture grade water can be substituted).

14. Can the GenePORTER reagents be used for in-vivo experiments?

The GenePORTER and the GenePORTER 2 cells transfection reagents have been successfully demonstrated to enhance gene expression in various mouse models including direct muscular injections and intratumoral injections. Follow the standard protocol for formulation of the DNA/GenePORTER complexes. Also, see Bogdanov, *et. al.* (2001) *Gene Therapy* 8, 515–522.

15. Are the GenePORTER reagents suitable for stable transfection?

The GenePORTER cell transfection reagents can be used for both transient and stable transfection. In order to achieve stable transfection, an antibiotic resistance gene must be transfected with the gene of interest. Selection medium added post transfection will select against the cells that have not incorporated the antibiotic resistance gene into their chromosomes. Please visit our website at www.genetherapysystems.com for information on high-expression vectors optimized for stable transfection

16. Can the GenePORTER reagents be used for co-transfection of more than one plasmid? Yes. The GenePORTER cells transfection reagents can deliver multiple plasmids into cells. The specified conditions in the protocols are for total DNA quantity. Thus, it does not make any difference whether 1ug of DNA consists of 1 or 10 plasmids.

17. Can the GenePORTER reagents be used on quiescent (non-dividing) cells?

Yes. However, it is more difficult to achieve high transfection efficiencies with non-dividing cells.



18. Is there any “auto-fluorescence” associated with the GenePORTER reagents?

Similar to other polyvalent cationic lipids, the GenePORTER and GenePORTER 2 reagents have intrinsic fluorescent characteristics that may be visualized under fluorescent microscopy. However, this auto fluorescence is usually minimal, and typically a camera must be set to a long exposure time (e.g., ¼ sec.) to detect it. In contrast, GFP fluorescence is usually bright enough that it is necessary to set a short exposure time (e.g., 1/60 or 1/120 sec.) to avoid overexposure. At such short exposure times, auto fluorescence is usually not visible, so it is not a problem. However, if background fluorescence is visible, it is easily distinguishable from a GFP signal when proper controls for the experiment are done, i.e. mock transfection of cells using just the DNA or just the GenePORTER alone.

19. What are the precipitates that settled on top of the cells after adding the DNA/GenePORTER Lipoplexes, which are visible under light microscopy?

After adding the DNA/GenePORTER complex to the cells, there may be grainy sand-like precipitates settled on the tops of the cells. This is a common observation among all lipid-based transfection reagents. "Precipitates" on top of the cells are the actual DNA/GenePORTER complexes attached to the cell surface via electrostatic interaction. The uptake of the desired DNA could not be achieved without the complexes coming into direct contact with cell surface.

20. Can the GenePORTER/DNA complexes be saved for another day?

The GenePORTER / DNA complexes must be made fresh each time. It is specified in the protocol that the complexes must be used within 45 minutes. The complexes have the tendency to aggregate, enlarge, and precipitate out of solution if left for too long.

21. What is the difference between the BoosterExpress™ reagents and the DNA Diluents contained in the GenePORTER 2 kit? Are they comparable?

The BoosterExpress reagents are not comparable to the DNA Diluents included in the GenePORTER 2 kit. Their respective biological functions are very different. The DNA Diluents are designed to help compact the DNA so it may be effectively delivered into the cells by the GenePORTER 2 reagent. In contrast, the BoosterEXPRESS reagents are designed as cell culture media additives that boost gene expression. They do not increase DNA uptake by the cells, but rather enhance gene expression by stimulating the cell's metabolic machinery. The increase of gene expression with the BoosterExpress reagents is cell type dependent.

22. Can I use BoosterExpress with my own transfection reagent?

Yes. Even though the BoosterExpress reagents are optimized for use with the GenePORTER Transfection reagents, they have been shown to significantly increase transgene expression when used in combination with a variety of commercially available transfection reagents.

23. In what buffer and at what concentration should my DNA be suspended prior to diluting it with serum-free media (or DNA diluents for GenePORTER 2)?

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 reaction sizes.

