

BioPORTER[®] Reagent Instruction Manual

Catalog Numbers: BP502401, BP509604



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OVERVIEW

Kit Contents and Ordering Information

The BioPORTER[®] protein delivery reagent is the latest innovation at Gene Therapy Systems, Inc. It is a unique lipid-based formulation that allows the delivery of proteins, peptides or other bioactive molecules into a broad range of cell types.

The BioPORTER reagent is provided in a dried form and needs to be resuspended in methanol or chloroform before use according to section 1.1 on page 7.

Catalog Number	Number of Tubes	Description	Size or Amount
BP502401	1	BioPORTER [®] Reagent, dried.	24 reactions (6 well plates or 35 mm dish) or 96 reactions (24 well plates)
	1	β -galactosidase control protein.	10ug at 100 μ g/ml
	1	FITC-antibody control protein (fluorescein-labeled goat IgG)	10ug at 100 μ g/ml
BP509604	4	BioPORTER [®] Reagent, dried.	4 x 24 Reactions (6 well plates or 35 mm dish) or 4 x 96 reactions (24 well plates)
	1	β -galactosidase control protein.	10ug at 100 μ g/ml
	1	FITC-antibody control protein (fluorescein-labeled goat IgG)	10ug at 100 μ g/ml

Use the contents of the table above to determine the appropriate catalog number for your needs. You can order the products above by contacting us at:

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Stability and Storage

The BioPORTER reagent is shipped frozen. Upon receipt and for long-term use, store all reagent tubes at -20°C . BioPORTER reagent is stable at least for 1 year at the recommended storage temperature.

Introduction

We congratulate you on your purchase of the BioPORTER[®] reagent, a new, versatile and efficient reagent for intracellular delivery of bioactive molecules, such as proteins, peptides or antibodies, into a broad range of cell types.

Although there are many effective reagents available to introduce transcriptionally active DNA into viable cells, approaches to deliver functional peptides and proteins into living cells are limited. For this reason, Gene Therapy Systems, Inc. has investigated a unique protein delivery approach using a lipid-based carrier system. The resulting BioPORTER reagent is a novel composition that contains a proprietary reactive lipid mixed with other components.

This new approach is easy to use and more economical than both microinjection and electroporation for delivering biologically active proteins into living cells. The specific formulation of the BioPORTER reagent can deliver various molecules over a broad range of cell types in serum-free conditions. The delivery is fast and the optimum delivery is usually reached after 3 to 4 hours of incubation. Various molecules such as fluorescent-antibody, high and low molecular weight dextran sulfate, phycoerythrin-BSA, β -galactosidase, caspase 3, caspase 8 and granzyme B have been successfully delivered into the cytoplasm of a variety of different adherent and suspension cells with BioPORTER reagent. Furthermore, apoptotic proteins such as granzyme B, caspase 3 or caspase 8 delivered into cells with the BioPORTER reagent are functional, since they can drive cells into apoptosis.

Now you can make your macromolecules directly available for a variety of studies like intercellular signaling, cell cycle regulation, control of apoptosis, study of oncogenesis, and transcription regulation to name a few. Gene Therapy Systems, Inc. has extensively tested BioPORTER reagent and verified its effectiveness in delivering active molecules into a wide variety of cells. BioPORTER reagent is a new and powerful tool in the functional genomics and proteomics arsenal. The BioPORTER reagent is:

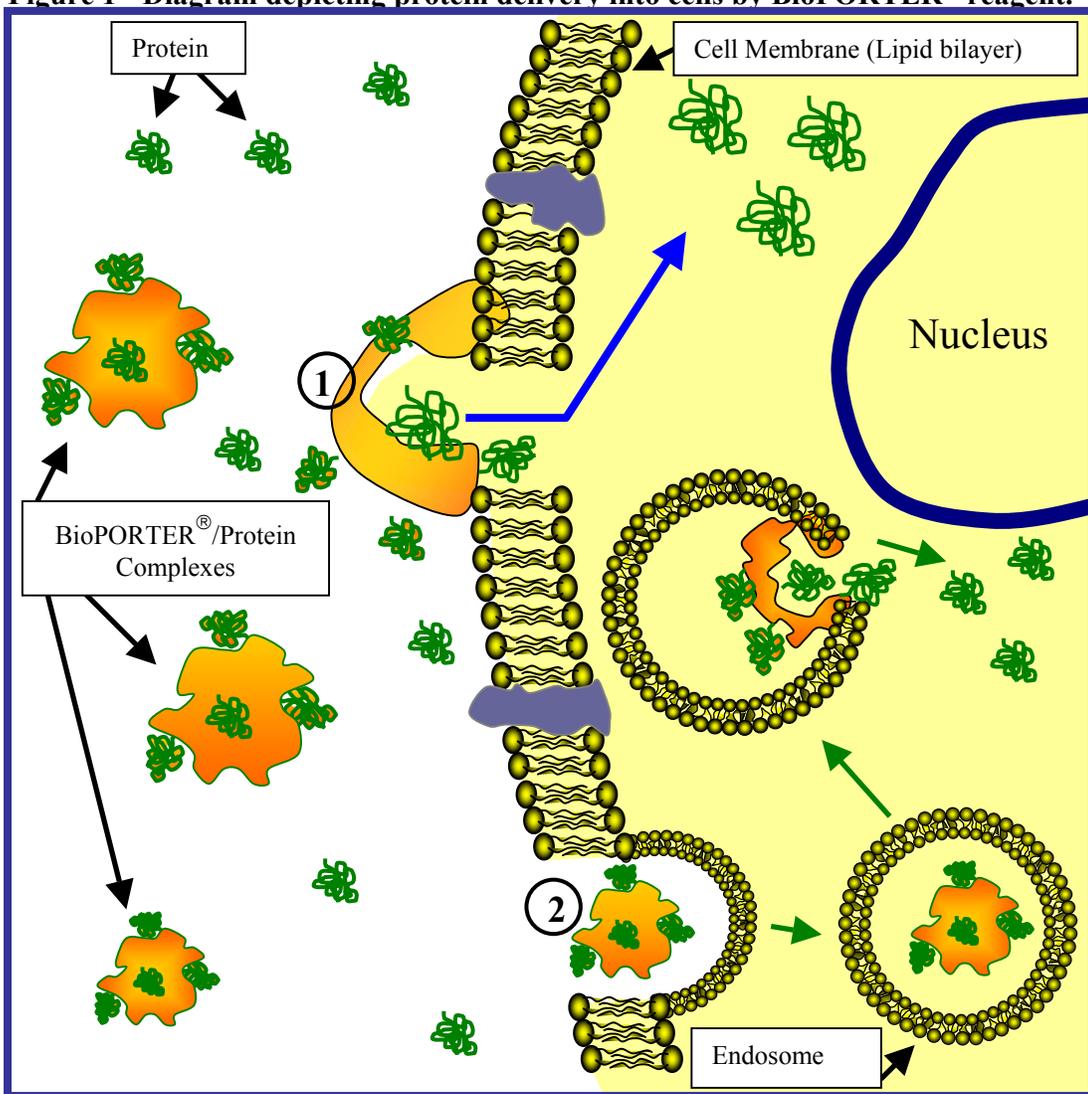
- Economical and easy to use
- Effective in multiple cell types (Table 1)
- Non-cytotoxic
- Fast and efficient. Optimum delivery obtained 3 to 4 hours after incubation
- A non-covalent complex
- Stable with an extended shelf life at -20° C.

HeLa-S3	BHK-21	293	CHO-K1
NIH 3T3	CV-1	B16-F0	COS-1
K562	COS 7	Jurkat	Ki-Ras 267 β 1
HepG2	MDCK	HeLa	P19

Summary of the BioPORTER[®] Protein Delivery Mechanism

The dried BioPORTER reagent formulation is first dissolved in a solvent and aliquoted into small eppendorf tubes according to the type of assays conducted (see Methods and Procedures). After complete drying, BioPORTER is formulated with a solution of the protein or peptide to be delivered. The BioPORTER reagent reacts quickly and interacts non-covalently with the protein, peptide or other molecules creating a protective vehicle for immediate delivery into cells. The hydrated mixture is then added onto cells, and the BioPORTER/protein complexes attach to negatively charged cell surfaces. The BioPORTER reagent can then fuse directly with the plasma membrane and deliver the captured protein into the cells (see ① in Figure 1), or the BioPORTER/protein complexes are endocytosed by the cells and then fuse with the endosome releasing the BioPORTER-captured protein into the cytoplasm (see ② in Figure 1). Delivery of molecules with the BioPORTER reagent is very easy and requires only 4 hours of incubation with the target cells.

Figure 1 - Diagram depicting protein delivery into cells by BioPORTER[®] reagent.



METHODS AND PROCEDURES

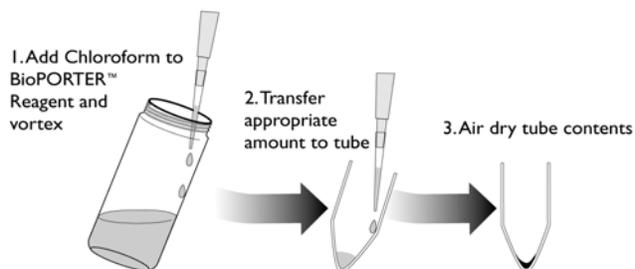
1. General Protocol

NOTE *The conditions that follow are recommended as good starting guidelines only. For best performance of the BioPORTER[®] reagent, we recommend that you optimize component concentration, cell number, time of incubation, and protein hydration buffers. Because optimum conditions are cell type and assay-dependent, we provide you with optimization guidelines in the Appendix section on page 13.*

1.1. Preparation of the BioPORTER[®] Reagent

1.1.2. Dissolve each tube containing the dry film of BioPORTER reagent with 250 μ l of methanol or chloroform. Vortex for 10-20 seconds at top speed before each use.

Summary diagram of BioPORTER Preparation Process



CAUTION *Handle chloroform with caution by wearing the appropriate protective clothing and using a well ventilated space or hood. To avoid excessive evaporation of chloroform keep containers closed as much as possible.*

1.1.3. Pipette the desired amount of BioPORTER reagent into an eppendorf tube (see table below for suggested amount). Be sure to dispense the BioPORTER solution to the bottom of the tube. The amount of BioPORTER reagent needed varies depending on the type of experiment you are conducting (cell type, assay sensitivity, plate size, etc.). We recommend that you start by using 2.5 μ l of BioPORTER reagent per reaction in a 24-well plate or 10 μ l for a 6-well plate and vary the amount of protein to be delivered. We highly recommended that you optimize the delivery conditions by varying the amount of protein/peptide to be delivered first and then varying the amount of BioPORTER reagent, if necessary. Further optimization guidelines are offered in the Appendix section on page 13.

Table 2 - Suggested Volume of BioPORTER[®] reagent for Various Assays

Tissue Culture Dish	BioPORTER [®] Volume (μ l)	Number of reactions/kit
96-well	1	240
24-well	2.5	96
12-well	5	48
6-well	10	24
60mm dish	20	12
100mm dish	35	7

- 1.1.4. Leave the eppendorf tubes containing the BioPORTER reagent under a laminar flow hood to evaporate the solvent for at least **2 hours** at room temperature. For larger volumes, evaporate for at least 3-4 hours.

Alternatively, you can use an inert gas like argon or nitrogen to gently and quickly evaporate the solvent by blow-drying. To avoid splashing the BioPORTER reagent, apply the gas flow gently and gradually. Make sure that the reagent stays at the bottom of the tube.

- 1.1.5. **Optionally**, you may vacuum the BioPORTER dry film for 1-2 additional hours to completely remove any trace of solvent.

IMPORTANT

The quality of the delivery reaction is severely affected by the presence of methanol or chloroform in the solution. Make sure that you follow the 2-4 hours recommendation for drying to assure the removal of all traces of solvent before adding your protein or peptide.

NOTE

We recommend that you aliquot the BioPORTER reagent immediately after dissolving in 250 μ l of chloroform. This will help prevent extensive evaporation of the solvent and causing variations in reagent concentration. Dried BioPORTER reagent can be safely stored at -20° C for at least one year without any substantial loss of activity.

- 1.1.6. At this stage, you can proceed to the next step, preparation of the BioPORTER/protein complexes or store the tubes at -20° C until use.

1.2. Preparation of the BioPORTER[®]/Protein Complexes

- 1.2.1. Dilute the protein, peptide, or other molecules in one of the following buffers:

HBS (10 mM HEPES, 150 mM NaCl, pH 7.0)
PBS (20 mM Na phosphate, 150 mM NaCl, pH 7.4)
Tris Buffer (10 mM Tris, 150 mM NaCl, pH 7.0)

- 1.2.2. The final concentration of your proteins, peptides, or molecules of interest will vary according to their intrinsic properties and the type of assay performed. Further optimization guidelines are offered in the Appendix section on page 13. For the following molecules listed in Table 3 below, we have found that the following concentration ranges yielded good delivery results:

Summary Diagram of the BioPORTER/protein Preparation Process

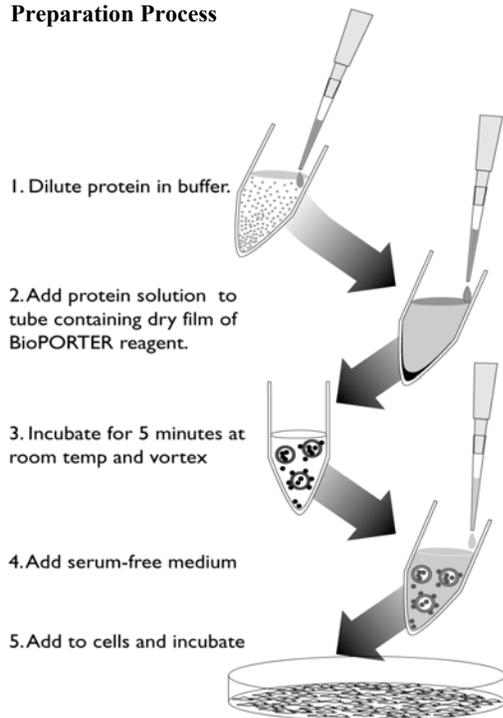


Table 3 – Protein concentration ranges used successfully for delivery	
Antibody, β-galactosidase or dextran sulfate	50-250 $\mu\text{g/ml}$
Caspase3	0.05 to 0.3 units/ μl (165 to 1000 pg/ μl)
Granzyme B	7.5 to 60 ng/ μl

IMPORTANT *Our experimental results suggest that some (though not all) highly positively charged molecules interact poorly with the BioPORTER[®] reagent and are therefore not delivered into cells efficiently.*

- 1.2.3. Use the diluted protein solution to hydrate the dried BioPORTER reagent. The amount of protein, peptide, antibody or other molecules to be delivered will depend on the type of experiment (cell type, assay sensitivity, plate size, etc.). See Table 4 below for suggested amounts.

Pipette up and down 3 to 5 times. Let stand at room temperature for 5 minutes then vortex gently and briefly (3-5 seconds) at low to medium speed.

Table 4 - Suggested Quantity of Proteins and Hydration Volumes

Tissue Culture Dish	Protein/ Ab, β-gal (μg)	Protein/ Caspase3 (ng)	Protein/ Granzyme B (μg)	Hydration Volume (μl) for BioPORTER[®]
96-well	0.1-0.25	0.25-0.5	0.01-0.05	10
24-well	1-2	2-4	0.075-0.5	10-25
12-well	2-4	4-8	0.15-1	25-50
6-well	5-10	10-20	0.3-2	50-100
60mm	10-20	20-40	0.5-3	100-400
100mm	25-50	50-100	0.75-4	250-500

- 1.2.4. Add serum-free medium to the BioPORTER/protein complex to bring the final delivery volume up to the amounts recommended in Table 5 below.

Table 5 - Suggested Cell Number and Transfection Volume

Tissue Culture Dish	Number of cells	Total Delivery Mix Volume
96-well	1-2 x 10 ⁴	100 μl
24-well	0.5-1 x 10 ⁵	250 μl
12-well	1-2 x 10 ⁵	500 μl
6-well	2-4 x 10 ⁵	1 ml
60mm dish	5-10 x 10 ⁵	2.5 ml
100mm dish	10-20 x 10 ⁵	5 ml

- 1.2.5. Aspirate medium from the cells to be tested, wash once with serum-free medium (optional) and then transfer the final delivery mix onto cells.
- 1.2.6. For **adherent cells**, directly add the BioPORTER/protein complexes (resuspended in serum-free medium) onto the washed cells. For **suspension cells**, first count the cells, centrifuge them at 1200 rpm for 5 minutes, and then resuspend them in serum-free medium. Adjust their concentration according to the size of your plate or dish and your transfection volume. Pipette the cell suspension into the tube of BioPORTER/protein mixtures and then transfer it to your well or dish.

- 1.2.7. Incubate for 3-4 hours at 37° C. If longer incubation time is required, add one volume of 20% serum-containing medium directly to the well or dish. It is not necessary to change the medium after the initial serum-free incubation. However, if incubation times that are longer than 24 hours are necessary, medium can be replaced as needed.

NOTE

The presence of serum in the first hours of incubation is inhibitory for delivery. Make sure that the first 3-4 hours of incubation are done in serum-free conditions followed by growth in serum-containing medium.

- 1.2.8. Proceed with your experiment for observation or detection assays. Cells can be fixed or can be observed alive.
- 1.2.9. Two positive controls are provided in the Kit. The fluorescein-antibody and β -galactosidase can be tested as described below in the example protocols.

2. Example Protocols

2.1. Delivery of a Fluorescent Antibody, β -galactosidase, or dextran sulfate (High and low Molecular Weights) for 24-well plates (or 22 mm cover slips).

- 2.1.1. Seed 0.5 to 1×10^5 cells per well in a 24-well plate or on a cover slip and let grow overnight
- 2.1.2. Pipette 2.5 μ l of BioPORTER[®] reagent (dissolved in 250 μ l of methanol or chloroform) into the bottom of each eppendorf tube. Evaporate the solvent under a laminar flow hood for 2 hours (or blow dry with an inert gas). Optionally, vacuum the tube for 2 hours to completely remove any trace amount of solvent. Proceed to the next step (2.1.4) or store the tubes at -20°C until use
- 2.1.3. Dilute 0.5-2 μ g of FITC-Ab, dextran sulfate, or β -galactosidase in 10 to 25 μ l of HBS or PBS. For β -galactosidase we recommend using PBS (buffer formulas available in section 1.2.1). The FITC-Ab and β -galactosidase control proteins provided in the kit are ready to use without further manipulation. Just thaw and mix them well before use.
- 2.1.4. Hydrate the BioPORTER[®] reagent dry film with 10-25 μ l of the diluted protein solution. Pipette up and down 3 to 5 times. Incubate at room temperature for 3-5 minutes, then vortex briefly and gently at low to medium speed for few seconds.
- 2.1.5. Bring the final volume of the BioPORTER/protein mixture to 250 μ l with serum-free medium.
- 2.1.6. For 24-well plates, aspirate the medium from the cells to be tested, wash once with serum-free medium (optional) and then transfer the BioPORTER/protein mixture directly onto the cells.

If cover slips are used, blot the cover slip dry and place it in a 35-mm dish. Transfer the BioPORTER/protein mixture directly onto the cells.

- 2.1.7. Incubate cells in a 5% CO₂ incubator at 37°C for 4 hours. Add 250 µl (1 volume) of 20% serum-containing medium directly to the 24 well if the incubation time needs to be longer than 4 hours.
- 2.1.8. For cover slips, add 1 to 2 ml of growth medium to the 35-mm dish containing the cover slip if the incubation time needs to be longer than 4 hours.
- 2.1.9. After the incubation, wash the cells twice with PBS and proceed with the appropriate assay.

Fluorescent microscopy: after washing, cells growing on cover slips are mounted directly onto a hanging drop slide with PBS. Living cells are then directly observed under a microscope. Alternatively cells can be fixed for observation.

β-galactosidase assay (X-Gal staining): for all of our assays we have used the Gene Therapy Systems X-Gal staining Kit (cat # A10300K), with the brief protocol on the left.

B-galactosidase Assay Sample Protocol

1. Aspirate medium 4 to 24 hrs after β-gal delivery.
2. Wash cells twice with PBS (500 µl).
3. Fix cells with the 1x fixing solution (250 µl) for 10 min at room temperature.
4. Prepare the staining solution.
5. Remove the fixing solution and gently wash cells 2 x with PBS (500 µl)
6. Add the staining solution (250 µl) and incubate 2 hrs to overnight at 37° C
7. Remove the staining solution. Wash cells with PBS and examine under a light microscope. Calculate percentage of stained cells if desired.

2.2. Delivery of a Fluorescent Antibody, β-galactosidase, or dextran sulfate (High and low Molecular Weights) for a 6-well plate or 35 mm dish.

- 2.2.1. Seed 2 x 10⁵ cells in 6-well plate and let grow overnight.
- 2.2.2. Pipette 10 µl of BioPORTER[®] reagent (dissolved in chloroform or methanol) into the bottom of each eppendorf tube. Evaporate the solvent as described in section 2.1.2 above.
- 2.2.3. Dilute 5-10 µg of protein in 50-100 µl of appropriate buffer as in section 2.1.3 above.
- 2.2.4. Hydrate the BioPORTER reagent dry film with 50-100 µl of the diluted protein solution. Pipette up and down 3 to 5 times. Incubate at room temperature for 5 minutes, then vortex briefly and gently at low to medium speed.
- 2.2.5. Bring the final volume of the BioPORTER/protein mixture to 1000 µl with serum free medium
- 2.2.6. Aspirate the medium from the cells to be tested, wash one time with serum-free medium (optional) and then transfer the BioPORTER/protein mixture directly onto the cells.
- 2.2.7. Incubate cells in a 5% CO₂ incubator at 37°C for 4 hours. If incubation time needs to be longer than 4 hours, add 1000 µl (1 volume) of 20% serum-containing medium directly to the well.

2.3. Delivery of granzyme B and caspase 3 into Jurkat or Ki-Ras-267 β 1 cells for 24-well plates.

- 2.3.1. For adherent cells such as Ki-Ras-267 β 1 (prostate cancer cell line) seed 0.5×10^5 in 24-well and let grow overnight. For Jurkat cells see section 2.3.6.
- 2.3.2. Pipette 2.5 μ l of BioPORTER reagent (dissolved in chloroform or methanol) into the bottom of each eppendorf tube. Evaporate the solvent as described above (section 2.1.2)
- 2.3.3. Dilute caspase 3 at 330-660 pg/ μ l or granzyme B at 15-45 ng/ μ l in HBS buffer. Buffer formula is available in section 1.2.2.
- 2.3.4. Hydrate the BioPORTER reagent dry film with 10 μ l of the diluted protein solution. Pipette up and down 3-5 times. Incubate at room temperature for 3-5 minutes; vortex briefly and gently at low/medium speed for few seconds.
- 2.3.5. For **adherent cells** such as Ki-Ras-267 β 1, bring the final volume of the BioPORTER[®]/protein mixture to 200 μ l with serum-free medium. Aspirate the medium from the cells to be tested, wash once with serum-free medium (optional) and then transfer the BioPORTER/protein mixture directly onto the cells.
- 2.3.6. For **suspension cells** such as Jurkat, count and pellet the cells, resuspend them in serum-free medium to 0.5×10^6 cells/ml. Add 200 μ l of the cell suspension to the BioPORTER/Protein mixture and then transfer the mix to a 24-well plate.
- 2.3.7. Incubate cells in a 5% CO₂ incubator at 37°C for 4 hours. Add 1ml of serum-containing medium directly to the well and incubate overnight.
- 2.3.8. The next day, proceed with the apoptosis assay using any commercially available annexin V-propidium iodine labeling kit. This assay can also be at time points earlier than 4 hours (see brief sample protocol on the left).

Apoptosis Assay Sample Protocol

1. Transfer medium and cells (after mild trypsinization for adherent cells) to 13 x 75mm plastic tubes. Wash wells with some serum-containing medium, pool them together and centrifuge at 1400 rpm for 5 minutes.
2. Wash cells with 500 μ l of cold PBS without disturbing the pellet, centrifuge at 1000 rpm for 3 minutes.
3. Resuspend cells in 100 μ l of cold annexin V binding buffer.
4. Add annexin V-FITC and propidium iodine (PI) to your samples and incubate at room temperature according to the instructions of the annexin V-PI labeling kit manufacturer.
5. Analyze your samples as soon as possible by flow cytometry or fluorescence microscopy.

APPENDIX

Protocol for Optimization

It is highly recommended to optimize your conditions in order to get the best performance for the BioPORTER[®] reagent. Several parameters can be optimized as follows:

- Amount of protein, peptide or other molecules to be delivered.
- Hydration buffer containing the diluted protein solution.
- Amount of BioPORTER reagent.
- Concentration of the protein solution during the preparation of the complexes.
- Hydration volume for BioPORTER reagent.
- Cell types and cell culture density.
- Time of incubation.

Many of these factors have been investigated at Gene Therapy Systems during the research and development of the BioPORTER reagent. We recommend that you optimize one parameter at a time using the suggested conditions in the Methods and Procedures section.

1. Start by using a fixed amount of the BioPORTER reagent, for example use 2.5 μ l of BioPORTER reagent per reaction in a 24-well plate.
2. Vary the amount of protein to be delivered. Use a standard buffer to do so, for example HBS or PBS. Depending on the sensitivity of the endpoint assay, a greater amount of protein and BioPORTER reagent may be required.
3. If further optimization is required, fix the concentration and amount of protein/peptide to be delivered and vary the quantity of BioPORTER reagent (see table below). The BioPORTER reagent interacts with your molecules of interest via hydrophobic and electrostatic interactions, and because each molecule will have different charge and hydrophobicity, the amount of BioPORTER reagent may need to be changed. Although BioPORTER reagent is not cytotoxic at the recommended concentrations, it may show some signs of cytotoxicity at higher reagent to cells concentration ratios.

Tissue Culture Dish	BioPORTER[®] range (μl)
96-well	0.25-1.5
24-well	1.25-5
12-well	2.5-7.5
6-well	5-15
60mm dish	15-30
100mm dish	25-45

4. After you identify the correct amount of BioPORTER reagent and protein to be used for the delivery reaction, you can then optimize the volume used to hydrate the BioPORTER dry film (step 1.2.3). To test this parameter, fix the protein amount and vary the hydration volume for BioPORTER[®] reagent (see Table 4 in section 1.2.3).
5. Different dilution buffers for your protein solution such as Tris, HBS, and PBS buffers can be tested. We have found that for some molecules the buffer composition may be critical. Indeed, with β -galactosidase the efficiency is very good with PBS buffer but not with Tris buffer. Whereas with dextran sulfate HBS is the right buffer. Also, apH may also be critical for some molecules because of their different charge and hydrophobicity; varying the pH may help interaction with the BioPORTER reagent.

6. At this point the cell number can also be optimized since the delivery efficiency may be sensitive to the confluency of the cells in culture.
7. Depending on the type of functional assay performed, shorter or longer incubation time may be necessary.

If aggregation of the BioPORTER/protein complexes occurs during optimization (seen as large glowing particles under the microscope), try one or any combination of the following recommendations:

- Briefly sonicate the BioPORTER/protein mixture.
- Increase the BioPORTER hydration volume.
- Lower the concentration of protein or molecule.
- Lower the amount of BioPORTER reagent.

Quality Control

To assure the performance of each lot of the BioPORTER reagent, we qualify each component using rigorous standards. The following assays are conducted to qualify the function and activity of each kit component in living cells

Kit Component	Quality Control Standard
BioPORTER reagent	<ol style="list-style-type: none"> 1. Efficient FITC-antibody delivery in NIH-3T3 cells. 2. Efficient β-galactosidase delivery efficiency in NIH-3T3 cells. 3. Induction of apoptosis in Jurkat cells using granzyme B and caspase3. Delivery efficiency is assayed by monitoring the percentage of cells that become apoptotic through flow cytometry. 4. Testing for absence of bacterial and fungal contaminants.
FITC-Antibody Positive Control	<ol style="list-style-type: none"> 1. Analysis by gel electrophoresis and measurement of fluorescence. 2. Testing for intracellular delivery by the BioPORTER reagent in NIH-3T3 cells.
β -galactosidase Positive Control	<ol style="list-style-type: none"> 1. Testing for intracellular delivery by the BioPORTER reagent in NIH-3T3 cells.

Troubleshooting Guide

Problem	Possible Causes	Recommended Solutions
Low delivery efficiency	Solubilization of BioPORTER reagent.	<ul style="list-style-type: none"> – Make sure you use chloroform or methanol to solubilize BioPORTER reagent. – Vortex vigorously.
	Aliquoting of the BioPORTER reagent.	Be sure to aliquot the BioPORTER reagent to the bottom of the eppendorf tube
	Drying of BioPORTER reagent.	<ul style="list-style-type: none"> – Use sufficient time to air dry or vacuum dry BioPORTER reagent. – If used inert gas to dry the reagent, make sure that BioPORTER reagent did not splash on the side of the tube.
	Amount of BioPORTER reagent.	Vary the amount of reagent as recommended in the optimization protocol.
	Protein/peptide concentration.	Titrate the concentration and the hydration volume of BioPORTER reagent.
	Hydration buffers.	Change the protein dilution buffer and/or the pH to improve the delivery
	Mixing of BioPORTER reagent and protein.	Allow the mixtures to form for at least 3 minutes. Mix well by pipetting up and down; do not vortex vigorously at this step.
	Charge of Molecules to be delivered.	Highly positively charged molecules are difficult to deliver with BioPORTER reagent. Modify the hydration buffer or pH to change the charge of the molecules.
	Unknown properties of molecules to be delivered.	Mix a fluorescent molecule or directly label the protein of interest in order to monitor delivery.
	Cell Density.	Use cells that are 50-60% confluent.
	Wrong medium used.	Make sure to use serum-free medium during the first hours of delivery.
	Improper storage.	BioPORTER reagent is very stable but long exposure to elevated temperatures may cause degradation. Store BioPORTER reagent at –20° C.
	Time of incubation.	Incubate BioPORTER/protein complexes with cells for at least 3-4 hours.
Type of cell line used.	Test BioPORTER reagent with the positive controls in parallel with cell lines that were successfully used (see Table 1 on page 5 for cell line suggestions).	
Aggregation	Amount of BioPORTER reagent used.	Too much reagent could cause aggregation; lower the amount of BioPORTER reagent if you think you used an excess amount.
	Evaporation of the BioPORTER™ stock solution.	Excessive evaporation of the dissolved BioPORTER™ reagent will change its concentration. Titrate down or use lower amounts of reagent.
	Storage of the BioPORTER/ protein complexes.	BioPORTER/protein complexes should be freshly prepared. If complexes have been prepared and stored for too long, aggregation may occur
Cytotoxicity	Excess BioPORTER reagent.	Decrease the amount of reagent used.
	Molecules delivered are toxic.	<ul style="list-style-type: none"> – Use the appropriate control reactions like cells alone, BioPORTER reagent alone, “control” or “safe” protein alone, and your molecule(s) of interest alone. – Check the purity of the molecule of interest to be delivered.
	Unhealthy cells.	<ul style="list-style-type: none"> – Check cells for contamination. – Thaw a new batch of cells. – Cells are too confluent or cell density is too low. – Check the culture medium (pH, kind used, last time changed, etc.) – Check materials used for proper function (culture plates, incubator temperatures, etc.)

For additional troubleshooting assistance, please contact our Technical Support Department:

Toll Free 888-428-0558 extension 1	Fax 858-623-9494	E-mail tech1@genlantis.com
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Quick Reference Protocol for Experienced Users

<p>General Protocol</p>	<p>Preparation of BioPORTER® Reagent</p> <ol style="list-style-type: none"> 1. Dissolve each BioPORTER reagent tube in 250 µl chloroform or methanol. 2. Vortex for 10 seconds at high speed. 3. Aliquot the appropriate volume of BioPORTER reagent to the bottom of eppendorf tubes 4. Evaporate the solvent in a cell culture hood for at least 2 hours. Optionally, vacuum the BioPORTER reagent film for 2 additional hours. <hr/> <p>Preparation of BioPORTER®/Protein Mix</p> <ol style="list-style-type: none"> 1. Dilute protein, peptide or molecules of choice in HBS or PBS buffer. Concentration depends on the molecules used. 50-250 µg/ml is suggested 2. Add diluted protein solution directly to BioPORTER dry film and mix by pipetting. Hydration volume depends on the plate or dish size (see Table 4 in 1.2.3) 3. Incubate at room temperature for 3-5 minutes 4. Vortex BioPORTER/protein mix briefly then bring volume up with serum-free medium to an appropriate level (see Table 5 in section 1.2.4). 5. Transfer the mixture onto cells. 6. Incubate for 4 hours. 7. Add serum-containing medium if cells continue to incubate longer than 4 hours.
<p>Example Protocols</p>	<p>β-Galactosidase or FITC-Ab delivery in a 24-well plate (22 mm cover slips)</p> <ol style="list-style-type: none"> 1. Seed 0.5-1 x 10⁵ cells per well in 24-well plate or on cover slips and let grow overnight. 2. Aliquot 2.5 µl of BioPORTER (dissolved in 250 µl of solvent) into an eppendorf tube. 3. Evaporate the solvent in a cell culture hood for at least 2 hours. Optionally, vacuum the BioPORTER reagent film for 2 additional hours. 4. Dilute 1 µg of protein in 10 µl of HBS (Ab) or PBS (β-Galactosidase) 5. Hydrate BioPORTER reagent dry film with 10 µl of the diluted protein solution and mix by pipetting up and down 3 to 5 times. 6. Incubate at room temperature for 5 minutes. 7. Vortex BioPORTER/protein complex briefly; bring up final volume to 250 µl in serum-free medium. 8. Blot dry coverslips and put in 35 mm dish, or for 24-well plates. Aspirate old medium. 9. Transfer BioPORTER/protein/medium mixture to cells. 10. Incubate cells in a 5% CO₂ incubator at 37° C for 4 hours. 11. Add serum-containing medium if incubation time needs to be longer than 4 hours. 12. After incubation, wash cells and proceed with the appropriate assay. <hr/> <p>Delivery of Apoptotic proteins (granzyme B, caspase 3 or caspase 8)</p> <ol style="list-style-type: none"> 1. Seed 0.5 x 10⁵ adherent cells per well in a 24-well plate and grow O/N. For suspension cells see step 7 below. 2. Pipette 2.5 µl of BioPORTER (dissolved in 250 µl of solvent) into an eppendorf tube. 3. Evaporate the solvent in a cell culture hood for at least 2 hours. Optionally, vacuum the BioPORTER reagent film for 2 additional hours. 4. Dilute caspase 3 at 330 pg/µl (0.1 units/µl) and granzyme B at 30 ng/µl in HBS. Use β-galactosidase as a negative control by diluting it to 0.1 µg/µl in PBS. 5. Add 10 µl of the diluted protein solution to BioPORTER reagent dry film and mix by pipetting up and down 3 to 5 times. 6. Incubate at room temperature for 3-5 minutes. 7. Vortex BioPORTER/protein complexes briefly then: <p>For adherent cells bring final volume to 200 µl with serum-free medium. Aspirate the medium from the cells to be tested and then transfer the BioPORTER/protein mixture directly onto the cells.</p> <p>For suspension cells count and pellet cells, resuspend in serum-free medium to 0.5 x 10⁵ cells/ml. Pipette 200 µl of cell suspension into BioPORTER/protein mixture then transfer to a 24-well plate.</p> 8. Incubate cells in a 5% CO₂ incubator at 37°C for 4 hours, then add 1-2 ml of 10% serum-containing medium directly to the well and incubate overnight. 9. The next day, proceed with the apoptosis assay.