BioPORTER®

Reagent QuikEase™ Single-Use Tubes

Genlantis
A division of Gene Therapy Systems, Inc.

Cat. #	Contents	Quantity
BP502424 (24 rxns.)	BioPORTER® Reagent, dried	24 tubes
	β-galactosidase control protein	10 µg (100 µg/ml)
	FITC-antibody control protein (fluorescein-labeled goat IgG)	10 µg (100 µg/ml)
BP509696 (96 rxns.)	BioPORTER® Reagent, dried	96 tubes
	β-galactosidase control protein	10 µg (100 µg/ml)
	FITC-antibody control protein (fluorescein-labeled goat IgG)	10 µg (100 µg/ml)

Shipping	Shipped on Dry Ice
Storage	Store cells at -20°C. Stable for 1 year

Related Products	Catalog #
BioPORTER® Protein Delivery Reagent, 24 reactions	BP502401
BioPORTER® Protein Delivery Reagent, 96 reactions	BP509604
BioPORTER FITC Antibody Control, 10 µg	ABFITC01
BioPORTER β-gal Control, 10 µg	BGALCP01

Introduction: The BioPORTER® Protein Delivery Reagent is an efficient and trusted reagent for intracellular delivery of bioactive molecules, such as proteins, peptides, and antibodies, into a broad range of cell types. Although there are many effective reagents available to introduce transcriptionally active DNA into viable cells, The BioPORTER Reagent was designed specifically for the delivery of functional peptides and proteins into living cells, using a unique lipid-based carrier system.

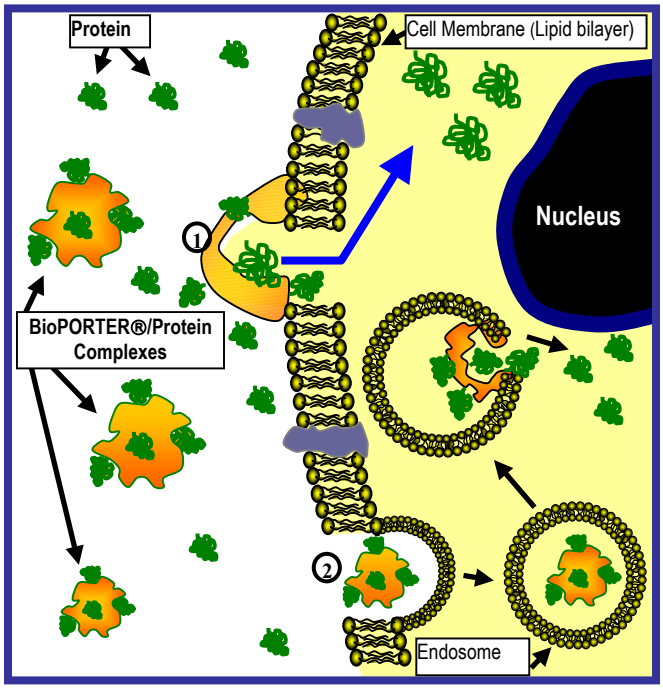
The BioPORTER Reagent is effective, easy to use, and more economical than both microinjection and electroporation for the delivery of biologically active proteins into living cells. The specific formulation of the BioPORTER Reagent can deliver various molecules, over a broad range of cell types, in serum-free conditions, and within 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 with the BioPORTER Reagent into the cytoplasm of a variety of different adherent and suspension cells†. Furthermore, apoptotic proteins such as granzyme B, caspase 3, or caspase 8 drove cells into apoptosis after delivery with the BioPORTER Reagent, confirming that BioPORTER delivers functional proteins into cells.

The QuikEase™ single-use tube kit format make it more convenient to use the BioPORTER Reagent. Each tube allows for one reaction in a 6-well dish. The BioPORTER QuikEase kits make it easier than ever to deliver your macromolecules directly for intercellular signaling, cell cycle regulation, control of apoptosis, study of oncogenesis, and transcription regulation studies to name a few.

† For a list of citations and cell types successfully used with the BioPORTER Reagent, visit our web site at www.genlantis.com.

Summary of the BioPORTER® Reagent 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, the BioPORTER Reagent 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 molecule, 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 the figure to the right), 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 the figure to the right). Delivery of molecules with the BioPORTER Reagent is very easy and requires only 4 hours of incubation with the target cells.



Genlantis
Telephone: (858) 457-1919 • (888) 428-0558 (U.S. Toll-free) • Fax: 858-623-9494 • www.genlantis.com

METHODS AND PROCEDURES

The conditions that follow are starting guidelines only. For best performance, we recommend optimizing component concentration, cell number, time of incubation, and protein hydration buffers. Further optimization guidelines are provided in the Optimization Guideline Section on page 4.

Table 1 – Suggested Number of Reactions For Each QuikEaseTube

Tissue Culture Plate-Type	Number of Reactions Per Tube
96-well	10
24-well	4
12-well	2
6-well	1

A. Preparation of the BioPORTER®-Protein Complexes

1. Dilute the protein, peptide, or other molecule 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)

2. The final concentration of your proteins, or molecules of interest will vary according to their intrinsic properties and the type of assay performed. Table 2 below shows a few concentration ranges that yielded good results:

   Table 2 – Protein Concentration Ranges Examples

Antibody, β-gal, or dextran sulfate	50-250 µg/ml
Caspase 3	0.05 - 0.3 units/µl (165 to 1000 pg/µl)
Granzyme B	7.5 to 60 ng/µl

3. The amount of protein or other molecules to be delivered will depend on the type of experiment (cell type, assay sensitivity, plate size, etc.) The following table offers some suggestions:

   Table 3 – Protein Amounts for Delivery (6-Well Plate)

Antibody, β-gal, or dextran sulfate	5-10 µg
Caspase 3	10-20 µg
Granzyme B	500-2000 ng

   If other tissue culture plates or dishes are needed, divide the protein amount per well by 2, 4, and 10 for 12-, 24-, and 96-well plates respectively.

   NOTE: Experimental results suggest that some, though not all, highly positively charged molecules are not efficiently delivered into cells because they interact poorly with the BioPORTER® Reagent (see Page 4 for suggestions).

4. Use 40 µl of the diluted protein solution to hydrate one QuickEase tube of BioPORTER Reagent. Hydration volume can vary between 20 and 100 µl according to your desired protein concentration. Pipette up and down 3-5 times. Let stand at room temperature for 5 minutes then vortex gently and briefly (3-5 seconds) at a low to medium speed.

5. Bring the final volume of the BioPORTER-protein mix to 0.5 ml with serum-free medium.

6. Aspirate medium from the cells to be tested, wash once with serum-free medium (optional) and then add the appropriate volume of serum-free medium to each well as indicated in Table 4 below. Transfer the appropriate volume of the BioPORTER-protein mix onto cells as in Table 4.

   Table 4 - Suggested Cell Numbers and BioPORTER®-Protein Mix Solution Volumes

Tissue Culture Plate Type	Number of Cells per Well	Serum-Free Medium Volume	BioPORTER -protein Mix Volume
96-well	1-2 x 10^4	50 µl	50 µl
24-well	0.5-1 x 10^5	125 µl	125 µl
12-well	1-2 x 10^5	250 µl	250 µl
6-well	2-4 x 10^5	500 µl	500 µl

7. For **adherent cells**, directly add the BioPORTER-protein complexes 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 as in Table 4 above. Adjust their concentration according to the size of plate used. Pipette the BioPORTER-protein mix into the tubes with cells and transfer all to plate wells.

8. Incubate for 3-4 hours at 37° C. If longer incubation time is required, add one volume of medium plus 20% serum directly to the cells. It is not necessary to change the medium up to 24 hours after the initial serum-free incubation. Replace medium as required for longer incubation times.
   NOTE: The presence of serum in the first hours of incubation inhibits protein delivery. Make sure that the first 3-4 hours of incubation is done in serum-free medium followed by growth in serum-containing medium.

9. Proceed with your experiment for observation or detection assays. Cells can be fixed or observed alive.

Telephone: (858) 457-1919 • (888) 428-0558 (U.S. Toll-free) • Fax: 858-623-9494 • www.genlantis.com

EXAMPLE PROTOCOLS

B. Delivery of a Fluorescent Antibody, β-Galactosidase, or Dextran Sulfate (High and low Molecular Weights) for 24-well Plates (or 22 mm Cover slips).

1. Seed 2-4 x 10^5 cells/well in a 6-well plate, or 0.5-1 x 10^5 cells/well in a 24-well plate (or on a cover slip) and grow overnight.
2. Dilute 4-8 µg of FITC-Ab., dextran sulfate, or β-galactosidase in 40 µl of HBS or PBS. For β-galactosidase, we recommend using PBS. The FITC-Ab. and β-galactosidase control proteins provided in the kit are ready to use without further dilution; just thaw and mix well before use.
3. Hydrate the BioPORTER® Reagent QuikEase dry film with 40 µl of the diluted protein solution. Pipette 3-5 times to mix. Incubate at room temperature for 3-5 minutes, then vortex briefly and gently at low speed for few seconds.
4. Bring the final volume of the BioPORTER-protein mixture to 500 µl with serum-free medium.
5. Aspirate the medium from the cells, wash once with serum-free medium (optional) and add the appropriate volume of serum-free medium to wells (see Table 4 above). Transfer the appropriate volume of the BioPORTER-protein mix onto the cells (see Table 4).

   For 6-well plates: plate directly; transfer the total volume of BioPORTER/protein complexes (0.5ml) to each well.
   For 24-well plates: transfer 125 µl of the mixture per well. Consequently, 4 wells can be assayed. Similarly, 2 and 10 wells can be tested for 12- and 96-well plates respectively.
6. Incubate cells in a 5% CO_2 incubator at 37°C for 4 hours. Add 1 volume of 20% serum-containing medium directly to each well if incubation time needs to be longer than 4 hours.
7. After the incubation, wash the cells twice with PBS and proceed with the appropriate assay:
   For fluorescent microscopy: after washing, cells growing on cover slips are mounted directly onto a hanging drop slide with PBS. Observe living (or fixed) cells under a microscope.
   For β-galactosidase assay (X-Gal staining): For best results, we recommend using the Genlantis X-Gal staining Kit (cat # A10300K); the protocol for this kit is available at www.genlantis.com.

C. Delivery of Granzyme B and Caspase 3 Into Jurkat or Ki-Ras-267 β1 Cells for 24-Well Plates.

1. For adherent cells such as Ki-Ras-267 β1 (prostate cancer) seed 0.5 x 10^5 in 24-well and let cells grow overnight. For Jurkat cells see Step 6 below.
2. Dilute caspase 3 to 330-660 pg/µl, or granzyme B to 15-45 ng/µl in HBS buffer (buffer recipes are in Step A.1 above).
3. Hydrate the BioPORTER Reagent QuikEase dry film with 40 µl 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 to medium speed for few seconds.
4. Bring the volume of the BioPORTER-protein mix to 0.5 ml with serum-free medium.
5. For **adherent cells** such as Ki-Ras-267 β1, aspirate the medium from cells, wash once with serum-free medium (optional), and then add 1254 µl of cells serum-free medium to the well. Transfer 125 µl of the BioPORTER-protein mixture directly onto the cells (enough for 4 wells of a 24-well plate).
6. For **suspension cells** such as Jurkat, count and pellet the cells, resuspend them in 125 µl serum-free medium at 8 x 10^5 cells/ml. Pipette 125 µl of the cell suspension, and then transfer the whole mix to a 24-well plate.
7. Incubate cells in a 5% CO_2 incubator at 37°C for 4 hours. Add 1ml of serum-containing medium directly to the well and incubate overnight.
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.

OPTIMIZATION GUIDELINES

It is highly recommended to optimize your conditions in order to get the best BioPORTER® Reagent performance. The following parameters can be optimized:

- Amount of protein or molecule to be delivered.
- Buffer used to dilute the protein.
- Amount of BioPORTER Reagent.
- Concentration of the protein solution.
- Hydration volume for the BioPORTER Reagent.
- Cell types and cell culture density.
- Time of incubation.

Optimize one parameter at a time as follows:

1. Start by using a fixed amount of BioPORTER: for example use one BioPORTER Reagent QuikEase tube per well (6-well plate) or ¼ of a QuikEase tube per well (24-well plate).
2. Vary the amount of protein to be delivered: Use a standard buffer, for example HBS or PBS. Depending on the sensitivity of the endpoint assay, a greater amount of protein may be required.
3. If further optimization is required: fix the concentration and amount of protein to be delivered, and then vary the quantity of the BioPORTER-protein mix transferred to cells (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.

NOTE: the BioPORTER Reagent is not cytotoxic at the recommended concentrations, but it may exhibit some cytotoxicity at higher reagent:cells concentration ratios.

Tissue Culture Plate	BioPORTER®-Protein Mix Volume Range (µl)
96-well	35-75
24-well	50-300
12-well	125-500
6-well	250-500

4. Optimize the volume or diluted protein used to hydrate the BioPORTER Reagent: do this after identifying the correct amounts of BioPORTER Reagent and protein to use in Step A.6. To test this parameter, fix the protein amount and vary the protein solution volume used to hydrate the BioPORTER® Reagent (see Table 4 in Step A.6).
5. Try different protein dilution buffers: Tris, HBS, and PBS, can be tested. For some molecules, the buffer used may be critical, for example PBS buffer works well with β-galactosidase, but not the Tris buffer. With dextran sulfate, HBS is the best buffer tested.
6. Try different buffer pH: pH may be critical for some molecules because of their different charge and hydrophobicity; varying the pH may improve interaction with the BioPORTER Reagent.
7. Optimize cell numbers: delivery efficiency may be sensitive to the confluency of the cells in culture.
8. Vary incubation times: depending on the type of functional assay performed, shorter or longer incubation time may influence delivery efficiency.

Telephone: (858) 457-1919 • (888) 428-0558 (U.S. Toll-free) • Fax: 858-623-9494 • www.genlantis.com

APPENDIX

Quick Reference Protocol for Experienced Users

General Protocol	**Preparation of BioPORTER®-Protein Mix** 1. Dilute protein of choice in HBS or PBS buffer. Concentration depends on the molecules used. 50-250 µg/ml is suggested. 2. Add 40 µl of the diluted protein solution directly to BioPORTER dry film and mix by pipetting. 3. Incubate at room temperature for 3-5 minutes 4. Vortex BioPORTER-protein mix briefly; then add 0.5 ml of serum-free medium. 5. Transfer the appropriate volume of the mixture onto cells (see Table 4 in Step A.6). 6. Incubate for 4 hours. 7. Add serum-containing medium if cells continue to incubate longer than 4 hours.
Example Protocols	**β-Galactosidase or FITC-Ab Delivery in a 24-well Plate (22 mm Cover Slips)** 1. Seed 0.5-1 x 10^5 cells per well in 24-well plate or on cover slips and let grow overnight. 2. Dilute 4-8 µg of protein in 40 µl of HBS (Ab.) or PBS (β-Galactosidase). 3. Hydrate BioPORTER Reagent dry film with 40 µl of the diluted protein solution and mix by pipetting up and down 3 to 5 times. 4. Incubate at room temperature for 5 minutes. 5. Vortex BioPORTER-protein complex briefly; bring up final volume to 500 µl in serum-free medium. 6. Blot dry coverslips and put in 35 mm dish, or for 24-well plates, aspirate old medium and add 125 µl of serum-free medium to cells. 7. Transfer 125 µl of the BioPORTER-protein-medium mixture to each well. 8. Incubate cells in a 5% CO_2 incubator at 37ºC for 4 hours. 9. Add serum-containing medium if incubation time needs to be longer than 4 hours. 10. After incubation, wash cells and proceed with the appropriate assay.
	Delivery of Apoptotic Proteins (Granzyme B, Caspase 3, or Caspase 8) 1. Seed 0.5 x 10^5 adherent cells per well in a 24-well plate and grow overnight. For suspension cells see Step 7 below. 2. Dilute caspase 3 to 330 pg/µl (0.1 units/µl) and granzyme B to 45 ng/µl in HBS. Dilute β-gal to 0.1 µg/µl in PBS for negative control. 3. Add 40 µl of the diluted protein solution to BioPORTER Reagent dry film and mix by pipetting up and down 3 to 5 times. 4. Incubate at room temperature for 3-5 minutes. 5. Vortex BioPORTER-protein complexes briefly then bring up to a volume of 500 µl with serum-free medium, then: for adherent cells bring final volume to 500 µl with serum-free medium, aspirate medium from the cells, add 125µl of serum-free medium to the cells, and then transfer 125 µl of the BioPORTER-protein mix directly onto the cells (enough for 4 wells). for suspension cells count and pellet cells, resuspend in serum-free medium to 8 x 10^5 cells/ml. Pipette 125 µl of the BioPORTER/protein mix to 125 µl of the cell suspension and then transfer it to a 24-well plate. 6. Incubate cells in a 5% CO_2 incubator at 37ºC for 4 hours; add 1-2 ml medium + 10% serum directly to wells; incubate overnight. 7. The next day, proceed with the apoptosis assay.

Troubleshooting Guide

Problem	Possible Causes	Recommended Solutions
Low delivery efficiency	Protein/peptide concentration	• Titrate the concentration and the hydration volume of the BioPORTER Reagent.
	Hydration buffers	• Change the protein dilution buffer and/or the pH to improve the delivery.
	Mixing BioPORTER and protein	• Allow mixtures to form for at least 3 minutes. Mix well by pipetting (do not vortex at this step).
	Charge of molecules to be delivered	• Highly positively charged molecules are difficult to deliver; modify the hydration buffer or pH.
	Unknown properties of molecules	• 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 stable, but exposure to elevated temperatures may cause degradation.
	Time of incubation	• Incubate BioPORTER-protein complexes with cells for at least 3-4 hours.
	Type of cell line used	• Use positive controls along with cell lines tested successfully (visit www.genlantis.com for a list).
Aggregation	BioPORTER-protein mix not fresh	• The BioPORTER-protein complexes should be freshly prepared, otherwise aggregation may occur.
	High amount of protein	• Lower the concentration of the amount of the protein to be delivered.
	Other	You may try the following recommendations regardless of the reason for aggregation: • Briefly sonicate the BioPORTER-protein mix; • Increase the volume of the protein solution used to hydrate the BioPORTER QuikEase tube; • Lower the concentration of the protein or biomolecule used.
Cytotoxicity	Excess BioPORTER reagent	• Decrease the amount of BioPORTER Reagent used.
	Molecules delivered are toxic	• Use the appropriate control reactions (cells alone, BioPORTER Reagent alone, control protein alone) along with your test protein, and check the purity of the molecule of interest to be delivered.
	Unhealthy cells	• Check cells for contamination or use 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.)