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ProductInformation

BioPORTER® QuikEase™ Protein Delivery Kit

Product Codes **BPQ24 and BPQ96**
Storage Temperature –20°C

TECHNICAL BULLETIN

Product Description
The BioPORTER Reagent is the latest innovation in macromolecule delivery technology. It is a unique lipid-based formulation that allows for the intracellular delivery of proteins, peptides, and bioactive molecules such as enzymes and antibodies, into a broad range of cell types. The BioPORTER® QuikEase™ kits contain 24 (BPQ24) or 96 (BPQ96) individual tubes of the BioPORTER Reagent in a convenient, ready-to-use, single reaction format. Each tube contains sufficient material of the lyophilized BioPORTER Reagent to perform 1 reaction in a 6-well plate (or 35 mm dish), 4 reactions in a 24-well plate (or 16 mm wells), and 10 reactions in a 96-well plate.

BioPORTER Reagent encapsulates macromolecules making them directly available to cells, thus becoming a valuable tool in a variety of studies including intercellular signaling, cell cycle regulation, control of apoptosis, study of oncogenesis, and transcription regulation. The BioPORTER protein transfection reagent has been extensively tested to verify its effectiveness in delivering **active** molecules into a wide variety of cells lines (see Table 1). The new BioPORTER QuikEase kit is an effective and powerful tool for functional genomics and proteomics studies. The BioPORTER QuikEase kit has been developed to provide a unique and highly efficient protein delivery approach using a proprietary lipid-based carrier system.

The BioPORTER Reagent is easy to use and more economical than either microinjection or electroporation for delivering biologically active proteins into living cells. The specific formulation of BioPORTER can deliver various molecules over a broad range of cell types in serum-free conditions. Molecule delivery is fast and reaches optimum levels after 4 hours of incubation. Various molecules (fluorescent-antibody, high and low molecular weight dextran sulfate, caspase 3, caspase 8, phycoerythrin-BSA, β-galactosidase, and granzyme B) have been successfully delivered into the cytoplasm of a variety of adherent and suspension cells with the BioPORTER Reagent. Furthermore, apoptotic proteins like granzyme B, caspase 3 or caspase 8 that were delivered into cells remained functional and induced apoptosis in cultured cells.

BioPORTER Protein Delivery Mechanism
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 cells (see **1** in Figure 1), or the BioPORTER/protein complexes are endocytosed and then fuse with endosomes releasing the BioPORTER-captured proteins into the cytoplasm (see **2** in Figure 1).

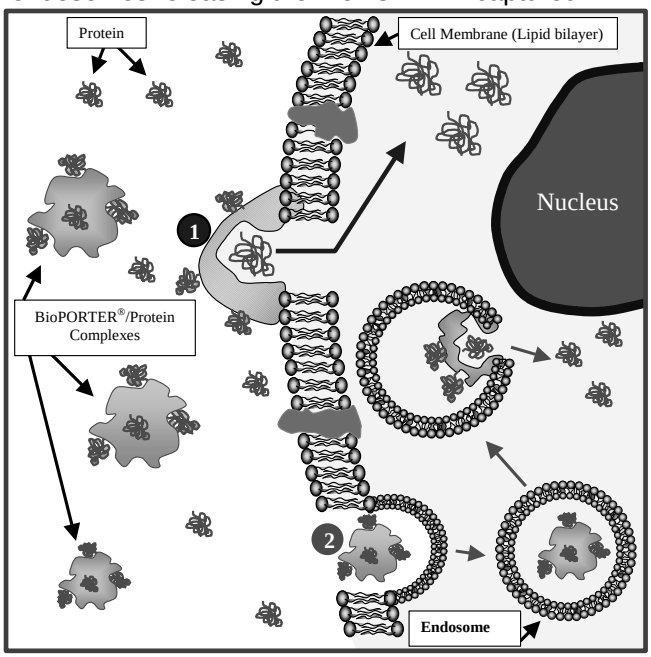

Figure 1: BioPORTER Protein Delivery Mechanism

Table 1 Cell Types Successfully Tested with BioPORTER QuikEase Kit			
HeLa	HeLa-S3	Jurkat	CV-1
*HEK-293	HepG2	Ki-Ras 267 β1	K562
NIH 3T3	P19	***CHO-K1	COS-1
B16-F0	**MDCK	BHK-21	COS 7

* HEK-293 Serum Free Medium, Product No. G 9916
**MDCK Serum-free Medium, Product No. M 3803 (growth) or M 3678 (maintenance)
***CHO-K1 Serum-Free Medium, Product No. C 5467

Kit Components

BPQ24 BioPORTER QuikEase Kit, 24 reactions

B 7558	BioPORTER Reagent Coated Reaction Tubes	24
F 9802	FITC-Antibody Control*	10 µg at 100 µg/ml
G 1668	β-Galactosidase Control	10 µg at 100 µg/ml

BPQ96 BioPORTER QuikEase Kit, 96 reactions

B 7558	BioPORTER Reagent Coated Reaction Tubes	96
F 9802	FITC-Antibody Control*	10 µg at 100 µg/ml
G 1668	β-Galactosidase Control	10 µg at 100 µg/ml

*Fluorescein-labeled goat IgG

Precautions and Disclaimer

BioPORTER QuikEase is a trademark of Gene Therapy Systems, Inc.
The BioPORTER Reagent and all of its components are sold for research use only. They are not to be used for clinical diagnostic or human use. All care and attention should be exercised in the handling of the kit components by following appropriate research lab practices.

Storage/Stability

The BioPORTER QuikEase kits are shipped frozen. Store all QuikEase tubes or kits at –20 °C.

Procedures

The conditions that follow are recommended only as starting guidelines. Optimization of component concentration, cell number, incubation time, and buffers is recommended for best performance of the BioPORTER Reagent. Optimum conditions are cell type- and assay-dependent. Optimization guidelines have been provided.

Experimental results suggest that some highly positively charged molecules interact poorly with the BioPORTER Reagent and are, therefore, not delivered into cells efficiently. However this is not a general rule since granzyme B (highly positively charged at neutral pH) is delivered effectively.

Each BioPORTER Reagent QuikEase tube was designed for one single use per well in a 6-well plate. If other tissue culture dishes are preferred, prepare the BioPORTER/protein complexes as recommended (see Table 2).

Table 2 Suggested reactions per tube	
96-well	10
24-well	4
12-well	2
6-well	1

The final concentration of the molecules of interest will vary according to their intrinsic properties and the type of assay performed. See the Assay Optimization section for additional information. Table 3 below lists suggested concentrations for various molecules.

Table 3 Protein Concentration Ranges Used Successfully for Delivery with BioPORTER	
FITC-Antibody (IgG)	50-250 µg/ml
β-Galactosidase	50-250 µg/ml
Dextran sulfate Product No. D 7037 or D 6924	50-250 µg/ml
Granzyme B Product No. G 9278	7.5 - 60 ng/µl
Caspase 3 Product No. C 1224	0.05 to 0.3 units/µl (165-1000 pg/µl)

Table 4 Protein Concentrations Used Successfully for Delivery (per well)			
Culture dish	IgG or β-Gal	Caspase 3	Granzyme B
6-well	5-10 µg	10-20 ng	500-2000 ng

Note: If other tissue culture dishes are preferred, the amount of protein per well can be divided by 2, 4, and 10 for 12-, 24- and 96-well plates respectively.

General Protocol

1. Dilute protein sample in suitable buffer:
   HBS, pH 7.0 (10 mM HEPES, Product No. H 4034 and 150 mM NaCl, Product No. S 5886)
   PBS, pH 7.4 (Product No. P 5886)
2. Add 40 µl of the diluted protein solution to hydrate one QuikEase tube containing the dried BioPORTER Reagent. Hydration volume can vary

between 20 and 100 µl according to the desired protein concentration.
3. Pipet up and down gently 3 to 5 times.
4. Incubate at room temperature for 5 minutes.
5. Vortex gently and briefly (3 to 5 seconds) at a low to medium speed.
6. Bring the final volume of the BioPORTER/protein mixture to 0.5 ml with serum-free medium.
7. Remove the medium from the cells to be tested.
8. Wash once with serum-free medium (optional) and add the appropriate volume of serum-free medium to the well (see Table 5).
9. Transfer the appropriate volume of the BioPORTER/protein mix onto cells (see Table 5).

Table 5 Suggested Cell Numbers and BioPORTER/protein Volumes/Well

Tissue Culture Dish	Number of Cells	Volume of Serum-free Medium	BioPORTER/ protein Mix
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

For adherent cells: directly add the BioPORTER/protein complexes (resuspended in serum-free medium) onto the cells.
For suspension cells: count cells and pellet by centrifugation at 1200 rpm for 5 minutes, then resuspend in the appropriate volume of serum-free medium (see Table 5 above). Adjust cell density suitable to culture plate. Pipet the BioPORTER/ protein mixtures into the tubes with cells and transfer all to wells/dishes.

10. Incubate for 3 to 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 up to 24 hours after the initial serum-free incubation. Replace medium as required for longer incubations times.*

Proceed with the experiment for observation or detection assays. Cells may be fixed or may be observed alive.

Example Protocol for the Delivery of Fluorescent Antibody, β-Galactosidase or Dextran sulfate

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 cover slips) and let grow overnight.
2. Dilute 4 to 8 µg of FITC-IgG, dextran sulfate, or β-galactosidase in 40 µl of HBS or PBS. For β-galactosidase, we recommend using PBS. The FITC-IgG and β-galactosidase provided in the kit are ready to use without further dilution. Thaw and mix positive controls well before use.
3. Hydrate one QuikEase tube with 40 µl of the diluted protein solution. Gently pipet up and down 3 to 5 times.
4. Incubate at room temperature for 3 to 5 minutes
5. Vortex briefly and gently at low to medium speed for a 3 to 5 sec.
6. Bring the final volume of the BioPORTER/protein mix up to 0.5 ml with serum-free medium.
7. Remove the medium from the cells.
8. Wash once with serum-free medium (optional) and add the appropriate volume of serum-free medium to wells (see Table 5).
9. Transfer the appropriate volume (see Table 5) of the BioPORTER/protein mix onto the cells.
10. Incubate cells at 37 °C for 4 hours. 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 up to 24 hours after the initial serum-free incubation.*

* The presence of serum in the first hours of incubation inhibits efficient delivery. Make sure that the first 3 to 4 hours of incubation occur in serum-free conditions followed by growth in serum-containing medium.

After incubation, wash the cells twice with PBS and proceed to assay:

- Fluorescent Microscopy: After washing, mount cells that are growing on cover slips 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 6-well plates) The following brief protocol may be adapted to several kits including Sigma Product Code: GAL-S or Gene Therapy Systems Cat. # A10300K)

1. Aspirate medium 4 to 24 hours after β-galactosidase delivery.
2. Wash cells twice with PBS (2 ml).
3. Fix cells with 1X fixing solution (1 ml) for 10 minutes at room temperature.
4. Prepare staining solution.
5. Remove fixing solution and gently wash cells 2 times with PBS (2 ml).
6. Add staining solution (1 ml) and incubate 2 hours to overnight at 37 °C.
7. Remove staining solution, wash cells with PBS and examine under a light microscope. Calculate the percentage of stained cells if desired.

Delivery of Granzyme B and Caspase 3 into Adherent or Suspension Cells

1. Seed 0.5 x 10^5 adherent cells/well (24-well culture plate) and culture overnight. For suspension cells, grow a culture overnight.
2. Dilute caspase 3 to 330 to 660 pg/µl and granzyme B to 15 to 45 ng/µl in HBS.
3. Hydrate one QuikEase tube with 40 µl of the diluted protein solution. Gently pipet up and down 3 to 5 times.
4. Incubate at room temperature for 3 to 5 minutes,
5. Vortex briefly and gently at low to medium speed for 3 to 5 sec.
6. Bring the final volume of the BioPORTER/Protein mix to 0.5 ml with serum-free medium.
7. For adherent cells (e.g. Ki-Ras-267 β1)
   Remove the medium from cells. Wash once with serum-free medium (optional). Add 125 µl of serum-free medium to the well. Transfer 125 µl of the BioPORTER/protein mix directly onto the cells (enough for 4 wells of a 24-well plate).
   For suspension cells (e.g. Jurkat)
   Count and pellet the cells. Resuspend them in 125 µl of serum-free medium at 8 x 10^5 cells/ml. Add 125 µl of the BioPORTER/Protein mix to the 125 µl of cell suspension. Transfer the whole mixture to a 24-well plate.
8. Incubate cells at 37 °C for 4 hours, then add 1 ml of serum-containing medium directly to the wells and incubate overnight.
9. Proceed with an apoptosis assay using an annexin V-propidium iodine labeling kit (e.g. Sigma's Apoptosis Detection Kit, Product Code APO-AF). This assay can also be done at earlier time points.

Following is a brief protocol for a common apoptosis assay:

1. Transfer medium and cells (after very mild trypsinization for adherent cells) to 13 x 75 mm 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 µl cold PBS without disturbing the pellet. Centrifuge at 1000 rpm for 3 minutes.
3. Resuspend cells in 100 µl of cold annexin V binding buffer.
4. Add annexin V-FITC and propidium iodine (PI) to the samples and incubate at room temperature according to the annexin V-PI labeling kit protocol.
5. Analyze samples as soon as possible by flow cytometry or fluorescence microscopy.

Assay Optimization

Optimization of reaction conditions is highly recommended in order to get the best BioPORTER Reagent performance. The following are the several parameters that can be optimized:

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

Many of these factors have been investigated during the development of the BioPORTER Reagent. Optimize one parameter at a time using the suggested conditions for best results.

- Use a fixed amount of BioPORTER Reagent, i.e. use one BioPORTER QuikEase tube per well (6-well plate) or 1/4 of a QuikEase tube of BioPORTER per well (24-well plate).
- Vary the amount of protein to be delivered. Use a standard buffer like HBS or PBS for dilutions. Depending on the sensitivity of the endpoint assay, a greater amount of protein may be required.
- If further optimization is required, fix the concentration and amount of protein/peptide to be delivered and vary the volume of BioPORTER/ protein mix transferred to cells (see Table 6 below). BioPORTER will interact with the molecules of interest via hydrophobic and electrostatic interactions and because each molecule will have different charge and hydrophobicity, the amount of BioPORTER may need to be changed. Although BioPORTER is not cytotoxic at the recommended concentrations, it may show some signs of cytotoxicity at higher reagent:cell ratios.

Table 6 BioPORTER/protein Mix Volume Ranges per Well

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

- Determine the correct amount of BioPORTER and protein to be used, then optimize the volume needed to hydrate the BioPORTER dry film with the protein solution. To test this parameter, fix the

protein amount and vary the hydration volume for BioPORTER (from 20 to 100 µl).

- Different protein dilution buffers like Tris, HBS, and PBS can be tested. For example, β-galactosidase delivery efficiency is very good with PBS but not with Tris; for dextran sulfate, HBS works best. pH may also be critical for some molecules because of their different charge and hydrophobicity. Varying the pH may help improve interaction with the BioPORTER Reagent.
- The cell density may also be optimized since Delivery efficiency may be sensitive to the degree of confluency of cells in culture.
- Depending on the type of functional assay performed, shorter or longer incubation times may be necessary.

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

- Briefly sonicate the BioPORTER/protein mix.
- Increase the BioPORTER Reagent hydration volume.
- Lower the concentration of protein or biomolecule used.

Troubleshooting Guide

Problem	Possible Causes	Recommended Solutions
Low delivery efficiency	Suboptimal protein/peptide concentration	Titrate the concentration and the hydration volume of BioPORTER.
	Suboptimal hydration buffers	Change the protein dilution buffer and/or the pH to improve the delivery.
	Insufficient mixing BioPORTER and protein	Allow the mixtures to form for at least 3 minutes. Mix well by pipeting up and down. **Do not vortex *vigorously* at this step**.
	Suboptimal amount of BioPORTER used	Vary the amount of reagent added onto cells (see the optimization protocol).
	Molecules to be delivered are highly charged	Highly positively charged molecules are difficult to deliver with BioPORTER. Modify the hydration buffer or pH to change the charge of the molecules.
	Unknown properties of the molecules to be delivered	Mix a fluorescent molecule or directly label the protein of interest in order to monitor delivery.
	Suboptimal 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 of the reagent. Store BioPORTER at –20 °C.
	Suboptimal incubation time	Incubate BioPORTER/protein complexes with cells for at least 3-4 hours.
	Type of cell line used is difficult to transduce	Test BioPORTER with the positive controls in parallel with cell lines that were successfully used (see Table 2 for cell line suggestions).
Aggregation	BioPORTER/ protein complexes not freshly prepared	BioPORTER/protein complexes should be freshly prepared. If complexes have been prepared and stored for too long aggregation may occur.
	High amount of protein used.	Too much protein or too high of a concentration can cause aggregation. Lower the concentration or the amount of protein to be delivered.
Cytotoxicity	Excess BioPORTER used	Decrease the amount of reagent used.
	Molecules delivered are toxic	Use the appropriate control reactions, e.g. cells alone, BioPORTER alone, safe or control protein alone, and compare to when formulated with the BioPORTER Reagent. Check the purity of the molecule of interest to be delivered.
	Unhealthy cells	Check cells for contamination. Thaw a new batch of cells. Cells may be too confluent or cell density may be too low. Check the culture medium (pH, type, last time changed, etc.). Check materials used for proper function (culture plates, incubator temperatures, etc.).

Quick Reference Protocol for Experienced Users

General Protocol	**Preparation of BioPORTER/Protein Mix** 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 40 µl of the diluted protein solution directly to BioPORTER dry film and mix by pipeting. 3. Incubate at room temperature for 3 to 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 5). 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-Antibody delivery in a 24-well plate (22 mm cover slips)** 1. Seed 0.5-1 x 10^5 cells in 24-well plate or on cover slips and let grow overnight. 2. Dilute 4 to 8 µg of protein in 40 µl of HBS (IgG) or PBS (β-Galactosidase) 3. Hydrate BioPORTER dry film with 40 µl of the diluted protein solution and mix by gently pipeting up and down 3 to 5 times 4. Incubate at room temperature for 5 minutes. 5. Vortex BioPORTER/protein complex briefly, then bring up final volume to 500 µl with serum-free medium. 6. Blot dry coverslips and put in 35 mm dish or for 24-well plates, remove old medium and add 125 µl of serum free medium to the cells. 7. Transfer 125 µl of the BioPORTER/protein/medium mixture to each well. 8. Incubate cells at 37 °C 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 in 24-well plates and culture overnight. For suspension cells see step 5 below. 2. Dilute caspase 3 at 330 pg/µl (0.1 units/µl) and granzyme B at 45 ng/µl in HBS. Use β-galactosidase as a negative control by diluting it to 0.1 µg/µl in PBS. 3. Add 40 µl of the diluted protein solution to the BioPORTER dry film and mix by pipeting up and down 3 to 5 times. 4. Incubate at room temperature for 3 to 5 minutes. 5. Vortex BioPORTER/protein complexes briefly then bring up final volume to 500 µl with serum-free medium. • For adherent cells bring the final volume to 500 µl with serum-free medium. Aspirate the medium from the cells to be tested, add 125 µl of serum free medium to the cells and then transfer 125 µl of the BioPORTER/protein mixture directly onto the cells (enough for 4 wells). • For suspension cells count and pellet the cells, resuspend them in serum-free medium at 8 x 10^5 cells/ml. Pipet 125 µl of the BioPORTER/protein mixture to 125 µl of the cell suspension and then transfer it to a 24-well plate 6. Incubate cells at 37 °C for 4 hours, then add 1 to 2 ml of 10% serum-containing medium directly to the well and incubate overnight. 7. The next day, proceed with the apoptosis assay

Record Keeping

Following is an example of a table that may be used to record all parameters necessary for efficient delivery of the biomolecule of interest using the BioPORTER Reagent (BP).

Protein Name	Buffer Name	Protein Amount	Protein Conc.	Hydration Volume	Cell Type	# Cells per Well	BP/protein mix volume	Incub. Time	Delivery Efficiency

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