BaculoPORTER™ Transfection Reagent

Instruction Manual

Catalog Numbers

T701007
T701035

UK & Rest of World
184 Milton Park, Abingdon
OX14 4SE, Oxon, UK
Tel: +44 (0) 1235 828 200
Fax: +44 (0) 1235 820 482

Switzerland
Centro Nord-Sud 2E
CH-6934 Bioggio-Lugano
Tel: +41 (0) 91 604 55 22
Fax: +41 (0) 91 605 17 85

Deutschland
Bockenheimer Landstr. 17/19
60325 Frankfurt/Main
Tel: +49 (0) 69 779099
Fax: +49 (0) 69 13376880

United States
23591 El Toro Rd, Suite #167
Lake Forest, CA 92630
Tel: + 1 800 987 0985
Fax: + 1 949 265 7703

amsbio
info@amsbio.com

TABLE OF CONTENTS

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The purchase price paid for the BaculoPORTER™ Transfection Reagent kit by end users grants them a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in the Kit Contents section). This kit is intended **for internal research only** by the purchaser. Such use is limited to the transfection of molecules into insect cells as described in the product manual. Furthermore, research only use means that this kit and all of its contents are excluded, without limitation, from 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").

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Product Use Limitations

The BaculoPORTER™ Transfection Reagent and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the handling of the kit components by following appropriate research laboratory practices.

OVERVIEW

Kit Contents

Catalogue Number	Number of Tubes	Description	Size or Aliquot
T701007	1 tube	Hydrated BaculoPORTER™ lipid	0.75 ml
T701035	5 tubes	Hydrated BaculoPORTER™ lipid	5 x 0.75 ml

Each BaculoPORTER™ Transfection Reagent tube contains sufficient material for 150 transfections based on transfecting 2 μg of DNA in a 35 mm tissue culture dish.

Shipping and Storage

The BaculoPORTER ™ Transfection Reagent is shipped at room temperature. For maximum stability, store all reagents at 4°C upon receipt. If stored properly, all components are stable for 6 months.

Related Products

For efficient transfection of DNA into a wide range of difficult-to-transfect cell types:

Product Name	Cat. No.	Quantity
GenePORTER® 2 Transfection Reagent	T202007	75 reactions (0.75 ml)
GenePORTER® 2 Transfection Reagent	T202015	150 reactions (1.5 ml)
GenePORTER® 2 Transfection Reagent	T202075	750 reactions (5 x 1.5 ml)

For efficient transfection of DNA into neuronal cells:

Product Name	Cat. No.	Quantity
NeuroPORTER® Transfection Reagent	T400150	1.5 ml
NeuroPORTER® Transfection Reagent	T400750	5 x 1.5 ml

For direct delivery of proteins into living cells:

Product Name	Cat. No.	Quantity
BioPORTER® Protein Delivery Reagent QuikEase™ Kit	BP502424	24 single use vials
BioPORTER® Protein Delivery Reagent QuikEase™ Kit	BP509696	96 single use vials

Introduction

The BaculoPORTER™ Transfection Reagent is a lipid formulation of a novel proprietary cationic lipid and dioleoyl phosphatidylethanolamine (DOPE) in a ratio targeted and optimized for the delivery of DNA into insect cells, especially Sf9 and Sf21. When mixed with DNA, the BaculoPORTER™ Transfection Reagent coats and interacts with the DNA to form a lipoplex of lipid and DNA. The formed lipoplex is efficient at delivering the target DNA into insect cells and with minimal toxicity.

The BaculoPORTER™ Transfection Reagent allows researchers to maximize the delivery of DNA combinations including: plasmids, transfer vector and baculovirus DNA, and bacmid DNA, into insect cells to optimize expression and maximize viral titer production. The BaculoPORTER Transfection Reagent offers the following benefits:

- Highly efficient delivery of plasmids, bacmids, and co-transfections of a transfer vector and baculovirus DNA into Sf9 and Sf21 cells.
- Simple and straightforward protocol.
- Compatibility with diverse insect cell culture medias.
- Minimized cytotoxicity.

When compared to other commercially available transfection reagents, the BaculoPORTER™ Transfection Reagent consistently offers superior transfection efficiencies and viral titer production.

METHODS AND PROCEDURES

1. Transfection of Bacmid DNA into Insect Cells

1.1. Set up the transfections in 35mm tissue culture dishes. Seed each plate with 900,000 cells in 2.0 ml of your preferred insect cell culture media (with or without serum). Allow the cells to incubate for 24 hours at 27°C.

1.2. Prepare the BaculoPORTER transfection mix as follows:

1.2.1. Prepare DNA Solution by combining 2.0 μg bacmid DNA and serum free media (SFM) for a total volume of 100 μl in a 1.5ml micro-centrifuge tube.

1.2.2. Prepare BaculoPORTER solution by combining 5.0 μl of BaculoPORTER Transfection Reagent with 95 μl SFM.

1.2.3. Combine the DNA and BaculoPORTER solutions to form the BaculoPORTER transfection mix; incubate at room temperature for 15 minutes to allow lipid-DNA complexes to form. Mix well by pipetting up and down several times.

1.2.4. Add 800 μl SFM to the tube, bringing the final volume of the BaculoPORTER transfection mix to 1.0ml.

1.3. Aspirate the media in which the insect cells are growing in (from Step 1.1 above) and wash the cells once with 2 ml SFM.

1.4. Aspirate the wash and overlay the cells with the 1.0 ml of BaculoPORTER transfection mix prepared in Section 1.2.3 above.

1.5. Incubate the cells for 4 hours in a 27°C incubator.

1.6. After incubation, aspirate the BaculoPORTER transfection mix and replace with 2.0 ml of your preferred insect cell culture media.

1.7. Incubate the transfected cells for 5 days at 27°C.

2. Co-transfection of Baculovirus DNA and Transfer Plasmid into Insect Cells

2.1. Set up the transfections in 35mm tissue culture dishes. Seed each plate with 900,000 cells in 2.0 ml of your preferred insect cell culture media (with or without serum). Allow the cells to incubate for 24 hours at 27°C.

2.2. Prepare the BaculoPORTER transfection mix as follows:

2.2.1. Prepare DNA solution by combining 0.5 μg of the baculovirus DNA and 2.0 μg of your transfer vector and serum free media (SFM) for a total volume of 100 μl in a 1.5ml micro-centrifuge tube.

2.2.2. Prepare BaculoPORTER solution by combining 5.0 μl of the BaculoPORTER™ Transfection Reagent with 95 μl SFM.

2.2.3. Combine the DNA and BaculoPORTER Solutions to form the BaculoPORTER transfection mix; incubate at room temperature for 15 minutes to allow lipid-DNA complexes to form. Mix well by pipetting up and down several times.

2.2.4. Add 800ul SFM to the tube, bringing the final volume of the BaculoPORTER transfection mix to 1.0ml.

2.3. Aspirate the media in which the insect cells are growing in (from Step 2.1 above) and wash the cells once with 2 ml SFM.

2.4. Aspirate the wash and overlay the cells with the 1.0 ml of BaculoPORTER transfection mix prepared in Section 2.2.3 above.

2.5. Incubate the cells for 4 hours in a 27°C incubator.

2.6. After incubation, aspirate the BaculoPORTER transfection mix and replace with 2.0 ml of your preferred insect cell culture media.

2.7. Incubate the transfected cells for 5 days at 27°C.

3. Transfection of Baculovirus Expression Plasmid into Insect Cells

3.1. Seed a tissue culture plate with insect cells at the appropriate densities, referred to in Table 1 below, using your preferred insect cell culture media. Allow the cells to incubate for 24 hours at 27°C.

TABLE 1: Seeding densities and transfection volumes for insect cells grown in different formats

Tissue Culture Dish	Cell Seeding Density per Well or Plate	DNA Amount to Use per Well or Plate (μg)	BaculoPORTER Amount to Use per Well or Plate(μl)	Final Transfection Volume per Well or Plate
96 well	15,000	1.0	1.0	100 μl
24 well	60,000	4.0	4.0	250 μl
6 well OR 35 mm	240,000	16.0	16.0	1 ml
60 mm	400,000	25.0	25.0	2.5 ml
100 mm	700,000	45.0	45.0	5 ml

3.2. Depending on the number of cells to be transfected, prepare the BaculoPORTER transfection mix as follows

3.2.1. Prepare the DNA Solution by combining the appropriate amount of the baculovirus expression plasmid with serum free media (SFM) up to half of the recommended transfection volume per well or plate.

3.2.2. Prepare the BaculoPORTER Solution by combining the appropriate amount of BaculoPORTER™ Transfection Reagent with serum free media (SFM) up to half of the recommended transfection volume per well or plate.

3.2.3. Combine the DNA and BaculoPORTER solutions to form the BaculoPORTER transfection mix; incubate at room temperature for 15 minutes to allow the lipid-DNA complexes to form. Mix well by pipetting up and down several times.

3.2.4. If needed, add SFM to the BaculoPORTER transfection mix to bring the final transfection volume up to the recommended value in TABLE I above.

3.3. Carefully aspirate the media in which the insect cells are growing in (from Step 3.1 above) and immediately wash the cells with PBS. Aspirate the PBS wash gently.

3.4. After washing the cells, gently add the BaculoPORTER transfection mix prepared in Section 3.2.3 above.

3.5. Incubate the cells for 4 hours in a 27°C incubator.

3.6. After incubation, aspirate the BaculoPORTER transfection mix and replace with an appropriate volume of your preferred insect cell culture media.

3.7. Incubate the transfected cells in a 27°C incubator. Heterologous gene expression can normally be detected after 24 hours.

APPENDIX

Troubleshooting Guide

Problem	Possible Causes	Recommended Solutions
Low transfection efficiency	Suboptimal BaculoPORTER/DNA ratio.	Optimize the BaculoPORTER/DNA ratio by trying any or all of the following ratios: 4:1, 2:1, 1:1, 1:2 BaculoPORTER to DNA (w/v).
	Suboptimal DNA concentration.	After establishing the optimal BaculoPORTER/DNA ratio, vary the quantity of DNA by decreasing or increasing few fold (2x and 4x for example) to optimize.
	Poor DNA quality.	Use DNase-free procedures for DNA isolation and purification. and plasticware. Check the DNA quantity and quality on an agarose gel and/or by using a spectrophotometer.
	Poor health of insect cells.	Thaw out a fresh aliquot of cells and passage once or a few times before transfecting. Avoid using cells that have been in culture or have been passaged for excessive periods of time.
	Suboptimal cell density.	Follow the recommendations for cell densities that are provided in the Materials and Methods Section. Alternatively, use cells that are 50, 70, or 80% confluent on the day of transfection.
	Improper storage.	The BaculoPORTER™ Transfection Reagent is very stable but long exposure to elevated temperatures and/or excessive freeze/thaw cycles may cause degradation of the reagent. Store the BaculoPORTER Transfection Reagent at 4° C.
	Wrong medium.	Be sure to use the correct serum-free insect cell culture media when forming the BaculoPORTER transfection mixes.
	BaculoPORTER/DNA transfection mixes are not freshly prepared.	The BaculoPORTER transfection mixes should be freshly prepared. If mixes have been prepared and stored for longer than 45 minutes, aggregation may occur and transfection efficiencies may be compromised.
Aggregation	Suboptimal BaculoPORTER /DNA ratio used.	Too much BaculoPORTER or too much DNA could cause aggregation; adjust the ratios as outlined above.
	BaculoPORTER frozen and thawed multiple times.	The BaculoPORTER Transfection Reagent is stable and should be stored at 4°C at all times. Avoid multiple freeze-thaw cycles that may cause lipid aggregation.
Cytotoxicity	Unhealthy cells.	- Check cells for contamination. - Thaw a new batch of cells. - Cells are too confluent or cell density too low. - Check culture medium (pH, kind used, last time changed). - Check materials used for proper function (culture plates, incubator temperatures, etc.).
	BaculoPORTER concentration too high	BaculoPORTER is a potent lipid delivery reagent; too much lipid however may cause toxicity, and if so, reduce the total amount of BaculoPORTER by 20 or 30% increments.