XpressBio

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BaculoFect

Catalog Number	Size
BF30-1	1.0 ml
BF30-2	2 x 1.0 ml
BF30-5	5 x 1.0 ml

Description:

BaculoFect is an aqueous formulation of positively charged lipids with a concentration of 1 mg/ml. It is designed for optimum liposome-mediated transfection of insect cells. Rates of efficiency with BaculoFect are much higher than in transfection of insect cells with other reagents such as Ca-phosphate or DEAE-Dextran.

Application: Transfection of insect cells with nucleic acids.

Formulation: Cationic lipids and co-lipids in water.

Assays: Up to 150 (6-well) or up to 75 (60 mm) per 1 ml reagent.

Shipping: Shipped at room temperature.

Storage: +4°C, DO NOT FREEZE

Stability: See label for expiration date.

Note: This product is for research use only. Not for use in human or animal diagnostics, therapeutics, or clinical applications.

General Guidelines

State of Cells

Cells to be transfected must be well proliferating and healthy (mid-log phase = 40 - 50% true confluency) with viabilities > 98%, doubling every 24 hours or less. Cells which have been in a quiescent state at confluency for a while (before seeding) may not be transfected as efficiently as cells which are growing rapidly. Therefore, it is recommended to use regularly passaged cells for transfection experiments. Microbial contamination, for example with mycoplasma or fungi, can drastically alter transfection results. The true confluency of the cells (adherent) to be transfected cannot be estimated visually with a microscope but can be optimally determined by means of a growing curve and comparison with counted cells. In many cases a 90-100% covered growing area is correlated with 40-50% true confluency.

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If cells are freshly thawed, remove any DMSO 2 hours before starting the transfection procedure. Do not use cells that have been in culture longer than 3 - 4 months. Over time their ability to be infected by viruses decreases even though they are viable and healthy. Maintain plates at 27°C in a non-CO_2 atmosphere. Keep cells moist throughout the procedure.

Quality of the Nucleic Acid

DNA or RNA should be of the highest purity if optimal results for transfection are desired. For example, endotoxins decrease transfection efficiency. Before its use in complex formation, DNA or RNA should not be stored diluted in medium for much longer than 5 minutes. Adsorption of DNA or RNA in container materials can result in decrease of transfection efficiency. Polypropylene shows a minimum tendency towards adsorption of transfection reagent and genetic material in comparison to glass and polyethylene.

Antibiotics

Antibiotics must be avoided where indicated. In some cases, cell death can be caused by use of antibiotics in the transfection medium. Do not use serum containing medium for lipid - DNA or RNA complex formation. The complex itself is stable towards serum. Therefore, the transfection can be carried out in the presence or absence of serum.

Optimization

BaculoFect shows a broad peak performance, but if optimal results are desired it is recommended to optimize the transfection protocol for each combination of plasmid and cell line used. Every cell line has a characteristically optimal DNA or RNA-lipid ratio. The format of dishes for lipoplex formation and cell culture can influence the ration and absolute amounts of reagents (likely caused by differing adsorption properties of the tube material based on differing surface dimensions).

Additionally, a protocol used for other transfection reagents should never be transferred to BaculoFect or any other different transfection reagent. Every transfection reagent possesses its own molecular structure with specific physical properties, which have an important influence on DNA or RNA-lipid ratios. Appropriate optimization instructions are given in this manual. As a rule, only little optimization is required if the recommended starting points are used.

Transfection Protocol

Co-transfection of Sf9 cells with BaculoFect

This protocol has been optimized for Sf9 cells and is a sample protocol for use of a baculovirus expression system (BES). Adaptation to customers' specific applications is recommended – DNA/lipid ration optimization means determining the optimum DNA amount to corresponding optimum amount of lipid.

Materials

- Sf9 cells (for each transfection use 2.0 - 3.0 x 10^6 cells per 60-mm Petri plate or adapted to any other plate size)

- Sf 900II (Invitrogen) insect medium or any equivalent medium like Grace's insect medium or ExCell 420 (JRH Biosciences)
- Optional fetal bovine serum
- 60-mm tissue culture grade Petri dishes (plates)
- Sterile glass, polystyrene or polypropylene tubes
- Linearized viral DNA at 0.5 µg/µl in TE (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)
- Recombinant transfer plasmid at 0.5 µg/µl in TE (if gene is to be expressed, cloned into Baculovirus Transfer Vector)
- Sterile pipette tips of various sizes
- Inverted microscope
- Rocking platform (not orbital)
- 27°C incubator

Procedure

1. For each transfection, seed 2.0 - 3.0 x 10^6 cells in a 60-mm Petri plate. After pipetting the cells into the plates, rock gently side to side to ensure an even monolayer (do not swirl the plates, this will result in cells clumping at the center).
2. Allow the cells to attach completely to the plate or flask (30 minutes – 4 hours at 27°C). Cells should be ~ 50% confluent when seeded at this density.
3. Verify that cells have attached by inspecting them under an inverted microscope. Now the cells are ready for use.
4. For each co-transfection, make the following transfection mixture in a 1.5 ml sterile polystyrene tube:
   Mix 0.84 µg linearized viral DNA and 4.2 µg recombinant transfer plasmid with serum and antibiotic-free medium to a total volume of 100 µl.
   Optional:
   Positive transfection control: Use 4.2 µg of a transfection control plasmid (coding ß-Galactosidase or ß-Glucuronidase) instead of recombinant transfer plasmid. This will produce plaques of successfully transfected cells by staining with X-Gluc or X-Gal.
   Negative transfection control: Use corresponding volume of medium instead of recombinant transfer plasmid. This will identify the background level of non-recombinants derived from any residual uncut nucleic acid.
5. For each transfection, mix serum and antibiotic-free medium with BaculoFect for a total volume of 100 µl. For optimization, a starting volume of 32 µl can be used.
6. Add DNA solution to BaculoFect solution immediately. Mix gently and incubate at room temperature for 15 – 20 minutes.
7. During incubation remove the medium from the cell dishes and rinse cells in the Petri plate(s) or flask(s) twice with 2 ml of antibiotic-free medium. Be careful to avoid disturbing the cell monolayer. Keep plates covered to maintain sterility and to prevent the monolayer from drying out. After the second washing very carefully add 2.5 ml of antibiotic-free medium to the cell monolayer (with or without serum).

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8. After completion of complex formation, add the transfection mixture to the cells. Add 2.5 ml of complete (serum and antibiotic containing) medium to the cells after 3 – 10 hours (optionally remove transfection solution in case of sensitive cells and add the new medium). Incubate 24 – 72 hours at 27°C.
9. Harvest the virus from the cell culture medium at 24 – 72 hours post-transfection.

Notes:

- The optimal exposure to the transfection mixture depends on the sensitivity of the transfected cell line. In case of high sensitivity, the transfection mixture should be removed and the cells should be washed twice prior to adding fresh medium and culturing for 72 hours (see step 8).
- Cells can be checked visually for successful transfection. Under an inverted microscope at 240 – 400 magnification, the viral gene can sometimes be observed as viral occlusions in transfected cells (crystals). In other cases, a positive sign of transfection is 25 – 50% increase of cell diameters and cell lysis.
- Virus plaque assay: The infectious potency of a baculovirus stock can be determined by examining and counting plaques in an immobilized monolayer culture. Many variants of this technique are used, depending on cell line, the nature of recombinant construct, and identification method. Commonly used identification methods are X-Gal, A-Gluc or Neutral Red staining. To visualize plaques and for protocols of plaque identification and staining, see instruction manuals of BES suppliers.

Optimization

Critical Optimization Parameters

Ratio of DNA or RNA to BaculoFect

The most important optimization parameter is the ratio of DNA or RNA to BaculoFect. For successful transfection a slightly net positive charge of the DNA- or RNA- BaculoFect complex is required. The optimal DNA- or RNA- BaculoFect ratio depends on the cell line. The amount of BaculoFect can be optimized within the range of 2-12 µl per µg nucleic acid. The maximum BaculoFect concentration should not exceed 35 µl/ml culture medium. The amount of nucleic acid can be optimized within a range of 1.0-3.0 µg referring to a 35 mm culture dish.

Quantity of transfection complex

In order to obtain the highest transfection results, optimization of the concentration of DNA- or RNA- lipid complex may be required.

Optimal ratio of DNA or RNA to BaculoFect and concentration of DNA or RNA-lipid complex may vary with the number of cells. An excessive amount of the complex can lead to over expression and/or lysis of cells (lipids are also lysis reagents). It is necessary to keep the number of seeded cells and incubation period constant until the transfection procedure for a reproducible optimization of these parameters.

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Effects of serum

To date, nearly all cell lines transfected with BaculoFect have shown superior results if transfection is performed in the presence of serum. Special cell lines may show different behavior. Transfection can be performed without serum, under serum reduced (e.g. 5%), or full serum (e.g. 10%) conditions.

There must be no serum present during **complex formation** between BaculoFect and DNA or RNA. Serum inhibits complex formation. Once the complex is formed, contact with serum is permitted.

Optimal ratio of DNA or RNA to BaculoFect and concentration of DNA- or RNA-lipid complex may vary with different serum concentrations.

Additional Optimization Parameters

Incubation time with transfection complex

Cells can be exposed to the transfection complex within a variety of time ranges between 3 – 72 hours. Generally, a transfection time range between 3 – 10 hours is sufficient. The best exposure to the transfection mixture is dependent on the sensitivity of the transfected cell line. With very sensitive and/or highly proliferating cells, the addition of fresh complete culture medium or replacement by such medium after transfection has favorable results.

Cell confluency

Good results are regularly obtained with a 90 – 100% covered growing area. In general, the transfection procedure should meet exponential growth phase of the cells because of the important role of cell division in transporting the DNA into the nuclease. Optimal confluency depends on the cell line used.

As a rule, confluency determined visually (“visual” confluency = percentage of growth surface covered with cells) is not identical with confluency determined by growing curve (= true confluency, cells growth inhibited). Best results are obtained if transfection is performed at the highest possible proliferation state (= 30 – 60% true confluency). This often corresponds to visual confluency of 90 – 100%.

Pluronic F68

Pluronic F68 or similar agents added to insect cell medium can optimize transfection results. These agents have stabilizing properties to sensitive insect cell membrane.

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Up and Downscale

Reagent quantities for different sizes of culture vessels are described. Proposed starting points for optimization are specified in parenthesis. As a rule, only minor optimization is required if the recommended starting points are used.

Proposed starting points for optimization in parenthesis.

1.		**Adherent cells (subculture the day before transfection. Incubate 18-24 h dependent on cell type)**			**Cells in suspension (day of transfection)**
	Culture dish diameter	35 mm	60 mm	100 mm	
		30-60% true (~ 90-100% visual) confluency on day of transfection			
	Cell number per dish* [x 106]	0.25–1.0 (0.5)	0.6–3.0 (1.2)	1.5–6.0 (2.5)	0.5 – 3.0 x 10^5/ml
	Culture medium volume per dish	1.5 ml	5 ml	12 ml	2-5 ml
2.	**Prepare INSECTOGENE/DNA or RNA-mixture**				
2a.	**Amount of nucleic acid**	1-3 µg (2.5)	2-6 µg (5)	3-9 µg (7.5)	2-6 µg (5)
	Final volume of nucleic acid / medium (free of serum + antibiotics) solution	50 µl	100 µl	150 µl	100 µl
2b.	**Amount of BaculoFect Transfection Reagent**	3-26 µl (16)	6-52 µl (32)	9-78 µl (48)	6-52 µl (32)
	Final volume of BaculoFect / medium (free of serum + antibiotics) solution	50 µl	100 µl	150 µl	100 µl
3a.	**Add the DNA or RNA-solutions (2a) into the BaculoFect (2b) solutions at room temperature (do not add BaculoFect to DNA or RNA solutions), pipette carefully up and down and incubate 15-20 minutes.**				
3b.	**Remove medium during complex formation and rinse cells with fresh medium (free of antibiotics, optionally with or without serum)**				
	Add volume of fresh medium:	~ 1.0 ml	~ 2.5 ml	~ 4.0 ml	
4.	**Add DNA or RNA-lipid complex solution to the cells and incubate 3-10 hours in an appropriate incubator**				
5.	**Subsequent exchange of the transfection medium with complete medium according to volumes given in Section 1.**				
6.	**Expression analysis after 24-72 hours (dependent on cell type and activity of the promoter used)**				

*Numbers of cells to seed depend on cell type and size. Optimization may be necessary. Maintain same seeding conditions between experiments.