

INSECTOGENE

The highly efficient Transfection Reagent for Insect Cells

For ordering information, SDS, publications and application notes see www.biont.com

Product	Order No.	Size
INSECTOGENE	T030-1.0	1.0 ml
INSECTOGENE	T030-2.0	2 x 1.0 ml
INSECTOGENE	T030-5.0	5 x 1.0 ml

Shipping: At room temperature

Storage: 4°C (*do not freeze*)

Stability: Best before: see label

Use: Only for research purposes *in vitro*; not intended for human or animal diagnostic, therapeutic or other clinical uses.

Description

The transfection reagent INSECTOGENE is an aqueous formulation of positively charged lipids with a concentration of 1 mg/ml. It is especially designed for optimum liposome-mediated transfection of insect cells. Rates of efficiency with INSECTOGENE are much higher than in transfection of insect cells with, for example, Ca-phosphate or DEAE-Dextran.

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1. General Guidelines

1.1 Specifications

Application	Transfection of insect cells with nucleic acids
Formulation	Cationic lipids and colipids in water
Assays	Up to 150 (6-well) or up to 75 (60 mm) per 1 ml reagent
Sterility	tested
Cell Culture	tested
Storage	4°C

1.2 Quality Control

Standard transfection assay. Absence of bacterial and fungal contamination is verified using thioglycolate medium.

1.3 Explanatory Remarks

Storage

INSECTOGENE is **delivered non-chilled and should be stored in a cooler at approx. 4°C immediately after receipt.**

Storage for a short period at room temperature is not a problem provided that the reagent is subsequently stored again at 4°C. Do not freeze!

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.

If cells are freshly thawed, remove any DMSO 2 h before starting 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-CO₂ atmosphere. Keep cells moist throughout the procedure.

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.

Quality of the nucleic acid

DNA or RNA should be of highest purity if optimal results for transfection are desired. After purification the nucleic acid should be free of traces of both caesium and endotoxins for example. Before use in complex formation DNA or RNA should not be stored diluted in medium for longer than 30 min. Adsorption of DNA or RNA (also lipid) by container materials can result in decrease of transfection efficiency.

Optimization

Although INSECTOGENE shows a broad peak performance, if optimal results are desired we recommend optimization of the transfection protocol for each combination of plasmid and cell line used.

Each cell line has its characteristically optimal DNA or RNA-lipid ratio; even the format of the dishes can influence the ratio or absolute amounts of reagents (probably caused by the 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 INSECTOGENE (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 the section "Optimization".

2. Working Instructions - General Standard Protocol -

		Adherent cells (subculture the day before transfection. Incubate 18-24 h dependent on cell type)			Cells in suspension (day of transfection)
1.	Culture dish diameter	35 mm	60 mm	100 mm	
		30-60% true (ca. 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 INSECTOGENE Transfection Reagent	3-26 µl (16)	6-52 µl (32)	9-78 µl (48)	6-52 µl (32)
	Final volume of INSECTOGENE / medium (free of serum + antibiotics) solution	50 µl	100 µl	150 µl	100 µl

3a.	Add the DNA or RNA-solutions (2a) into the INSECTOGENE (2b) solutions at room temperature (not contrariwise), pipette carefully up and down and incubate 15-20 min.			
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:	ca. 1.0 ml	ca. 2.5 ml	ca. 4.0 ml
4.	Add DNA or RNA-lipid complex solution to the cells and incubate 3-10 h 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 h (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.

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

3. Working Instructions

- Sample Protocol for Baculovirus Expression -

3.1 Co-transfection of Sf9 cells with INSECTOGENE

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 ratio optimization means determining the optimum DNA amount to corresponding optimum amount of lipid).

Materials

- Sf9 cells (for each transfection use $2.0 - 3.0 \times 10^6$ 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 $0.5 \mu\text{g} / \mu\text{l}$ in TE (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA)
- Recombinant transfer plasmid at $0.5 \mu\text{g}/\mu\text{l}$ 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 $2.0 - 3.0 \times 10^6$ 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 which will result in cells clumping at the centre!).
2. Allow the cells to attach completely to the plate or flask (30 min - 4 hours at 27°C). Cells should be ca. 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, makeup the following transfection mixture in a 1.5-ml sterile polystyrene tube:
Mix $x \mu\text{l}$ (total 0.84 μg) linearized viral DNA, $y \mu\text{l}$ (total 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 β -Galactosidase or β -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 100- $x \mu\text{l}$ serum- and antibiotics-free medium with $x \mu\text{l}$ of INSECTOGENE. 32 μl of transfection reagent can be used as starting volume for optimization.
6. Add DNA solution to the INSECTOGENE solution immediately, mix gently and incubate at room temperature for 15-20 min.
7. During incubation remove the medium from the cell dishes and rinse cells in the Petri plate(s) (or T-flask(s)) twice with 2 ml of antibiotics-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 2nd washing, very carefully add 2.5 ml of antibiotic-free medium to the cell monolayer (optionally with or without serum).
8. After completion of complex formation, add the transfection mixture to the cells. Add 2.5 ml of serum and antibiotic-containing (complete) 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.

Remarks

- 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 8.).
- Cells can be checked visually for successful transfection. Under an inverted microscope at 250-400 magnification, the viral gene can sometimes be observed as viral occlusions in transfected cells (crystals). In other cases, a positive sign of transfection is a 25-50% increase of cell diameters and cell lysis.
- Virus plaque assay: The infectious potency of a baculovirus stock solution can be determined by examining and counting plaques in an immobilized monolayer culture. Many variations of this technique are used, depending on cell line, nature of recombinant construct and identification method. Commonly used identification methods are X-Gal, X-Gluc or Neutral Red staining. To visualize plaques and for protocols of plaque identification and staining, see instruction manuals of BES suppliers.

4. Optimization

4.1 Critical optimization parameters

Ratio of DNA or RNA to INSECTOGENE

The most important optimization parameter is the ratio of DNA or RNA to INSECTOGENE.

For successful transfection a slightly net positive charge of the DNA- or RNA-INSECTOGENE complex is required. The optimal DNA or RNA to INSECTOGENE ratio depends on the cell line. The amount of INSECTOGENE can be optimized within the range of 2-12 µl per µg nucleic acid. The maximum INSECTOGENE 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.

An excessive amount of complex can lead to overexpression and/or lysis of cells (lipids are also cell membrane lysis reagents!).

Note: Optimal ratio of DNA or RNA to INSECTOGENE and concentration of DNA or RNA-lipid complex may vary with the number of cells. To ensure reproducible optimization of these parameters it is necessary to maintain a constant number of seeded cells and incubation period until transfection procedure.

Effect of Serum

A further key parameter is the effect of serum. Most of the cell lines transfected with INSECTOGENE showed superior results when transfection was performed in the presence of serum.

Nevertheless, some cell lines show diverging behavior. Accordingly, transfection can be performed without serum, under serum-reduced (e.g. 5%) or full-serum (e.g. 10%) condition.

Note: Presence of serum during **complex formation** between INSECTOGENE and DNA or RNA is **strictly forbidden** as serum inhibits complex formation. Once the complex is formed contact with serum is permitted.

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

4.2 Further 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. Usually 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 effects.

Time range between transfection and evaluation

Assay for gene activity should be performed 24-72 hours after the start of transfection. The optimal time is dependent on cell type, promoter activity and expression product (e.g. toxicity).

Cell confluency

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

Note: 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 is 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.

5. Miscellaneous

5.1 Important Information

This reagent is developed and sold for research purposes and *in vitro* use only. It is not intended for human or animal therapeutic or diagnostic purposes.

5.2 Warranty

Biontex guarantees the performance of this product until the date of expiry printed on the label when stored and used in accordance with the information given in this manual. If you are not satisfied with the performance of the product please contact Biontex Laboratories GmbH.

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