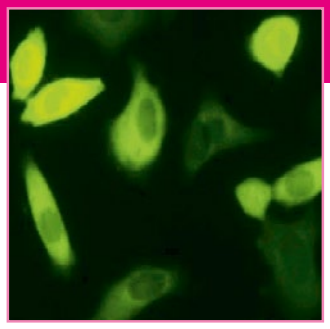
AppliCations
No.15

AppliFect – New Transfection Reagents



The term transfection describes the introduction of foreign genetic material into eukaryotic cells. Temporary introduction of DNA or RNA into the host cell is referred to as *transient transfection*, while the permanent integration into the genome is called *stable transfection*. Today, cationic lipid-mediated transfection is the most widely used method for gene transfer into eukaryotic cells in life science laboratory. AppliChem offers specialized reagents for all cell types and applications.

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Keywords

lipoplex

lipofection

gene transfer

recombinant expression

gene silencing

Introduction

Today physical, biological and chemical methods are applied to introduce genetic material into the cells.

1. Physical methods such as electroporation, microinjection or bioballistics ("gene gun") require special instruments. The application of such methods is often restricted to special cell lines or tissues. For instance, in majority of the cases the gene gun is used for plant cells, microinjection is a common tool to introduce RNA or DNA into *C. elegans* or Xenopus oocytes.
2. Biological systems for nucleic acid delivery employ viruses and protoplasts from bacteria. Viral transfection methods employ genetically modified viruses that are no longer pathogenic. Application of virus-mediated transfection is significantly limited by viral-related immunogenicity and the size limitation of the transgene. Both biological systems are highly evolved methods that are not easy to use for general applications.
3. The classical chemical method is calcium phosphate precipitation. This procedure involves co-precipitation of DNA associated with calcium phosphate crystals that are taken up by the host cells, unfortunately also resulting in noxious effects to most of the cells. As a result, the transfection efficiency is low, typically approx. 20 %. DEAE-Dextran is another commonly used chemical agent for transfection. Both of the methods work well for most permanent cell lines and may be appropriate if sufficient cell material is available to compensate for the typically low transfection rates.

The most widely applied transfection method is the chemical method of using cationic lipids, also called lipofection. The method is easy to use and does not require any additional lab instruments. State of the art lipid formulations have only low cytotoxic effects and often transfection rates of more than 80 % are achieved.

Transfection with cationic lipids

In aqueous solutions, cationic lipids and co-lipids form vesicles with a lipid bilayer, known as liposomes. When liposomes encounter nucleic acids they re-form into nucleic acid:lipid complexes called *lipoplexes*.

The postulated mechanism of DNA transfection involves four steps (Fig. 1).

1. Cationic lipids spontaneously form a so called lipoplex with negatively charged DNA.
2. Eukaryotic cells take up the lipoplex via endocytosis.
3. Inside the cell the endosomal structure is destroyed by increasing the osmotic pressure created by the lipids buffering action within the endosomes. Lipids of the lipoplex fuse with the endosomal membrane and DNA is released into the cytosol.
4. The barrier of the nucleus is permeable to foreign DNA only during cell division (Mitosis). A high rate of cell division thus ensures successful transfection and good gene expression.

The optimized formulation of AppliChem's AppliFect solutions positively affects crucial steps of the transfection mechanism: (i) in the formation of the lipoplex and (ii) in the release of DNA inside the cell. The rate of cell division is only little affected by AppliFect due to good compatibility. As a result the transfection efficiency is maximized.

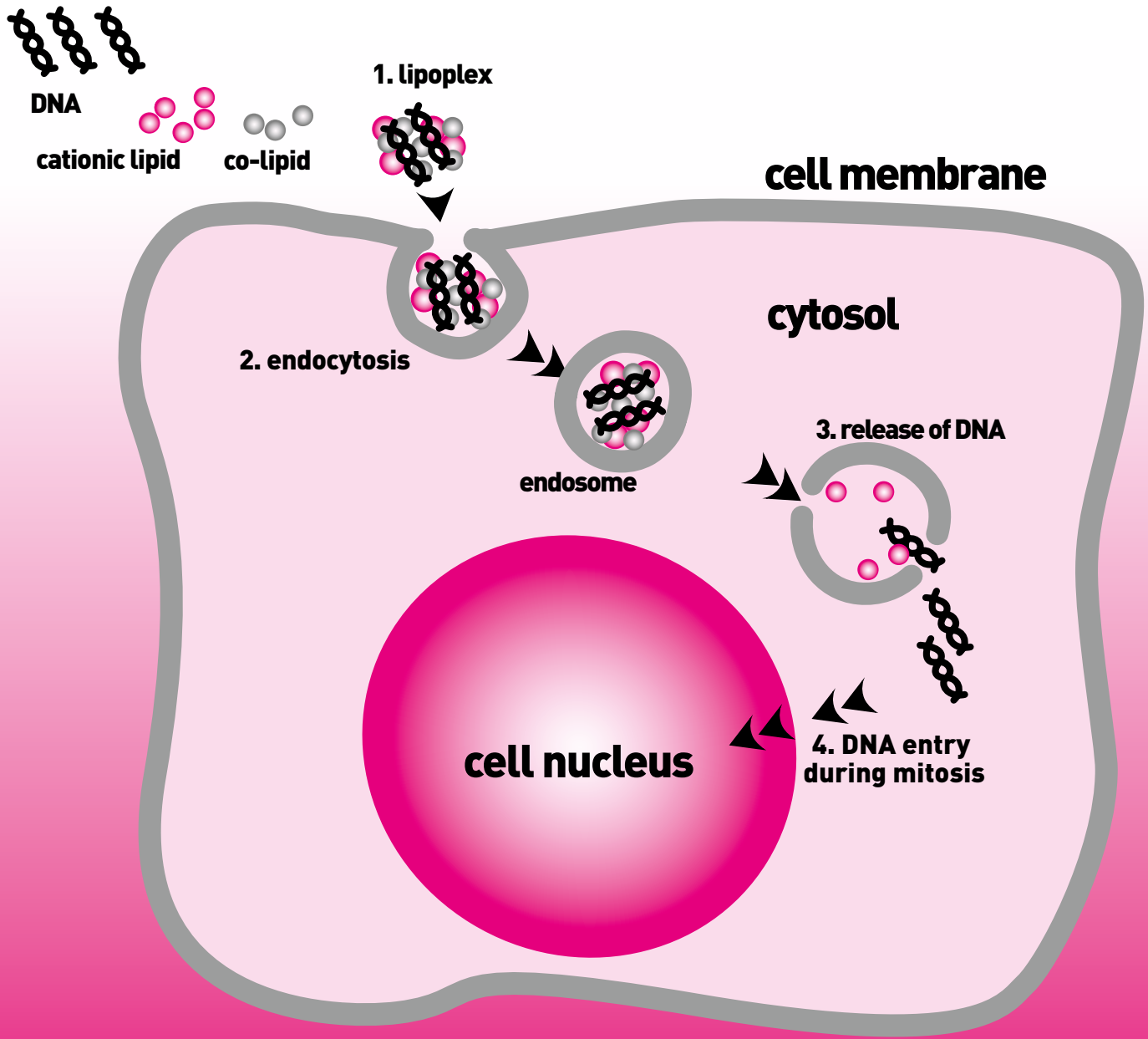

Fig. 1: The mechanism of DNA lipofection comprises formation of a lipoplex, endocytosis, DNA release from the endosome, and entry of foreign DNA into the nucleus during cell division.

The Range of AppliChem Transfection Reagents

AppliFect (A8886)

Polycationic transfection reagent for eukaryotic cells

AppliFect contains liposomes composed of a polycationic transfection reagent and a neutral co-lipid. The efficient uptake of DNA/RNA into eukaryotic cells is mediated by complexing of the nucleic acids with the liposomes of the transfection reagent. The transfected liposome-bound DNA/RNA is completely released within the cells, resulting in the maximum effect of transfected nucleic acids.

With AppliFect, highest expression levels, translation or inhibition of a gene can be achieved. The presence of serum does not interfere with the efficiency of transfections.

Examples of successfully transfected cell lines: 293T, CHO, COS-7, CV-1, HeLa, MDCK, NIH 3T3, Vero

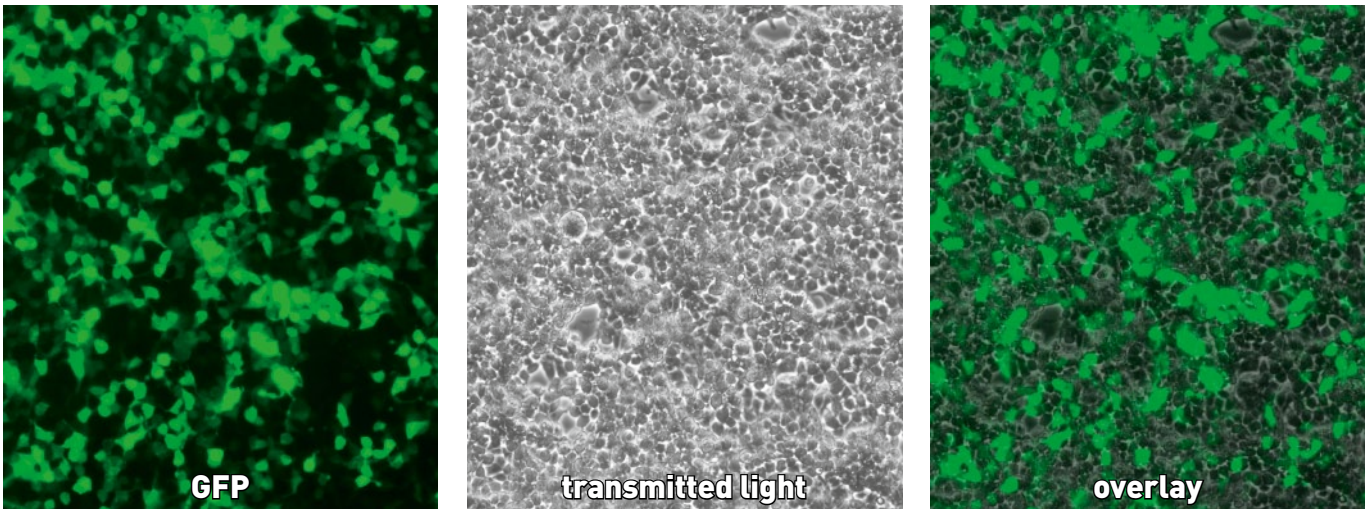

Fig. 2: Cos-7 cells transfected with GFP using AppliFect

AppliFect LowTox (A9027)

Transfection reagent for sensitive mammalian cells

AppliFect LowTox is an improved reagent for liposome-mediated transfection of mammalian cells. AppliFect LowTox achieves highest compatibility with many cell lines and highest transfection efficiency, while it shows only minimal cytotoxic effects. The transfected DNA or RNA is rapidly released in the cytosol from the lipoplex of cationic lipids and co-lipids.

The reagent shows no serum inhibition. AppliFect LowTox is the first choice for transfections of sensitive cell lines.

Examples of successfully transfected cell lines: 293T, BHK, CaCo-2, CHO-K1, COS-7, CV-1, HeLa, HepG2, J774, MDCK, NIH3T3, Vero

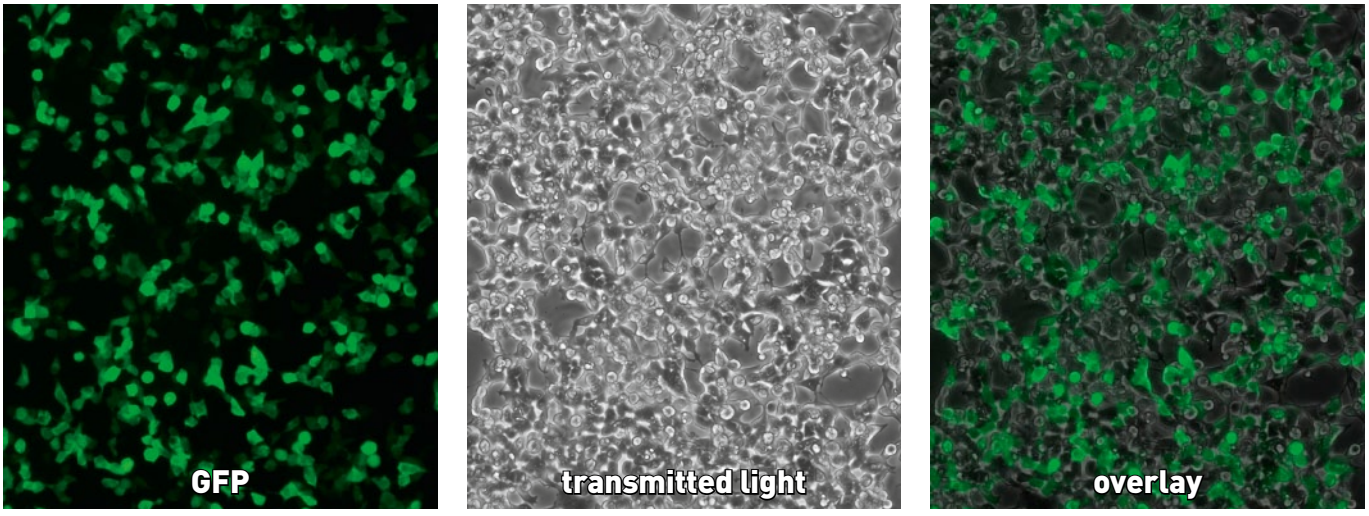

Fig. 3: 293T cells transfected with GFP using AppliFect LowTox

AppliFect SI (A8899)

Reagent for siRNA and miRNA transfection of mammalian cells

AppliFect SI is an advanced lipid formulation designed for the transfection of mammalian cells with siRNA and miRNA. Excellent knock-down results are achieved with even low amounts of RNA (in the picogram range). The AppliFect SI protocol for miRNA/siRNA transfection is easy to use and does not require any further optimization of the lipoplex composition since the optimal ratio of RNA to reagent is established for this product.

AppliFect Insect (A9002)

Reagent for the transfection of insect cells

AppliFect Insect is designed for optimum liposome-mediated transfection of insect cells. It is an aqueous formulation of positively charged lipids. Transfection rates achieved with AppliFect Insect are significantly higher in comparison to protocols using e.g. calcium phosphate or DEAE-Dextran. In addition, cytotoxic effects of AppliFect are considerably lower.

General Considerations for Successful Transfections

1. The cells should be proliferating and in good condition. We recommend regularly passaged cells for transfection. Microbial contaminants (such as by mycoplasma or fungi) will negatively affect the transfection.
2. Cell divisions support the transport of DNA into the nucleus. Therefore, DNA uptake during the exponential growth phase is critical for optimal results. Best transfection results are achieved when cells are at 30–60 % of "real" confluence. (This correlates with the "apparent" confluence of 90–100 % as seen through the microscope).
3. The DNA/RNA to be transfected should be of highest possible purity. For example, endotoxins significantly reduce the transfection efficiency.
4. Adsorption of DNA/RNA to the reaction tube surface can cause a decrease in transfection efficiency. Polypropylene reaction tubes are the best plastics for transfection procedures. Polypropylene has a low tendency to bind transfection reagent or genetic material in comparison to glass or polyethylene.

Stable Transfections

In contrast to transient transfections, cells are seeded at lower cell density. At the day of transfection cells should be less than 50 % confluent. After transfection, cell culture medium is replaced with an appropriate selection medium (including antibiotics).

Overview of AppliChem's Transfection solutions

Description	AppliFect	AppliFect LowTox	AppliFect SI	AppliFect Insect
Product code	**A8886**	**A9027**	**A8899**	**A9002**
Applications	plasmid DNA transfection, transient or stable transfection	plasmid DNA transfection, co-transfection of siRNA and plasmid DNA, transient or stable transfection	transfection of siRNA or miRNA for gene silencing	transfection of DNA or RNA (suitable for Baculovirus expression vector system, BEVS)
Cell types	eukaryotic cells, suspension and adherent cells	(sensitive) eukaryotic cells, suspension and adherent cells	mammalian cells	insect cells (e.g. Ag55, Anso, As43, Bm5, SF21, Mos-20, S2, SF9)
Technology	polycationic lipid reagent	formulation of cationic lipids and co-lipids	formulation of cationic lipids and co-lipids	cationic lipids
Features	inexpensive, economic suitable for a wide range of cells and applications,	minimal cytotoxicity high transfection efficiency	low cytotoxicity low (picogram) amount of RNA sufficient for full knock-down	no serum inhibition easy scale-up for production of recombinant proteins
Number of transfections/1 ml	ca. 500 24-well 200 6-well	ca. 500 24-well 200 6-well	ca. 1000 96-well 250 24-well	ca. 100 6-well 50 60 mm dish
Pack sizes available	A8886,00002 200 µl A8886,0001 1 ml	A9027,0001 1 ml	A8899,00002 200 µl A8899,0001 1 ml	A9002,00005 500 µl A9002,0001 1 ml

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