AppliFect

Polycationic *transfection reagent for eukaryotic cells*
Product code A8886

Description

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 cells, so that the maximum effect of transfected nucleic acids is obtained. 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. Therefore, the transfection reagent is especially suitable for sensitive cell types.
1 ml of the AppliFect solution is sufficient for 200 transfections on 35 mm cell culture dishes (6-well plates) or up to 70 transfections on 60 mm plates.
Order No.: A8886,00002 0.2 ml
A8886,0001 1 ml

Shipment: Ambient temperature

Storage: 2-8°C (Do not freeze!)

Contents

General Information

Storage
Soon after receipt store AppliFect in the refrigerator at 2-8°C (do not freeze!)
Short term storage and transport at room temperature does not interfere with the quality of the product.

Quality Control
Product quality is checked by a standardized transfection assay. Microbiological testing is performed to exclude contamination by bacteria or fungi.

Warranty
AppliChem can only guarantee for the described properties of the product if applied according to the product information. Please note the expiration date printed on the label. If you are still not satisfied with this product, please contact us at service@applichem.com

This product is exclusively for research and for in-vitro application. It should not be used for therapeutic and diagnostic applications in humans.

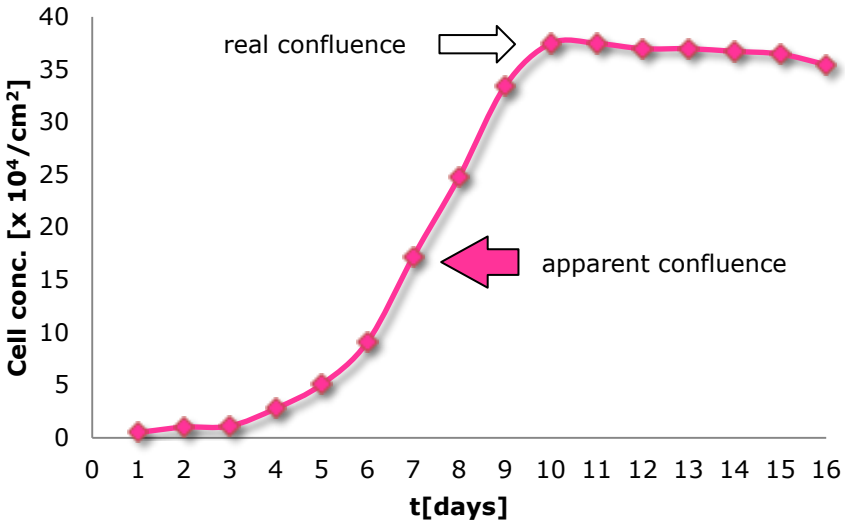
1.1 Properties of Cells for Transfection

For best transfection results the cells should be proliferating and in good and healthy condition. We recommend regularly passaged cells for transfection. (The transfection rate is usually low in cell cultures already grown confluent).
Microbial contaminants (such as by mycoplasma or fungi) will negatively affect the transfection.
In some cases the use of antibiotics in transfection medium leads to cell death. Antibiotics must be omitted in the steps indicated in this manual.

Confluence of Cells - Apparent and Real Confluence

The confluence of cells is usually determined by microscopy. The estimated "apparent" confluence does not reflect the maximum cell density (so-called "real" confluence). Best transfection results are achieved while cells are in the state of highly active proliferation – at 30-60% of real confluence. This correlates with the "apparent" confluence of 90 - 100%.

Cell divisions support the transport of DNA into the nucleus. Therefore, DNA uptake during the exponential growth phase is critical for optimal results of DNA transfection. The range of the exponential growth phase should be determined for each cell type individually.



A Typical growth curve of COS-7 cells. The apparent confluence (pink arrow) differs significantly from the real confluence (white arrow). Yet, the apparent confluence provides an important clue for the best time point for transfections.

1.2 General Guidelines for Optimization

We recommend optimization of the transfection protocol for each combination of plasmid and cell line. Individual cell lines exhibit optimal ratios of nucleic acid to lipid. Even the format of the cell culture plates and vessels used for the formation of the lipoplex will influence these ratios. In addition, the optimal quantities of reagents used may vary with the experimental set-up.

Protocols employing other transfection reagents cannot be transferred to AppliFect protocols. Each transfection reagent has its characteristic molecular structure with specific physical properties that have considerable influence on the nucleic acid: lipid ratios.

The DNA/RNA to be transfected should be of highest possible purity. For example, endotoxins significantly reduce the transfection efficiency. Adsorption of DNA/RNA to the reaction tube surface can cause a decrease in transfection efficiency. Thus, during transfection do not incubate DNA/RNA in serum-free medium longer than 5 minutes prior to the mixing with AppliFect-medium solution. 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.
Please see instructions for optimization in *chapter 3*.

1.3 Stable Transfection

Follow the general assay procedure. In contrast to transient transfections, seed the cells at lower cell density. At the day of transfection, cells should be less than 50% confluent. After transfection replace the medium with an appropriate selection medium (including antibiotics).

Protocols

For the first experiments use the starting values provided in the protocol (see *chapter 3*, Table 1 and 2). For optimization adjust amount of reagent and incubation times accordingly.

2.1 Standard protocol 1
Transfection of adherent cells (12-well format)

1. In 1 ml of complete medium seed 1.0 – 4.0 · 10^5 cells (standard 2.0 · 10^5) on a 12-well culture dish.

   Optimum cell densities depend on the cell type and cell size.

2. Incubate the cells for 18 – 24 h at 37°C in a CO_2-Incubator (until 90 – 100% of the culture dish is covered by the cells).

3. Bring DNA/RNA solutions as well as AppliFect to room temperature. Gently swirl the individual solutions.

4. Prepare the following solutions.

   Use polypropylene plastics preferentially, otherwise use glass or polyethylene tubes.

   Always pipette medium first in order to prevent direct contact of AppliFect and DNA/RNA solutions with the surface of the vials.

   Solution A: Add 0.5 – 1.5 µg DNA/RNA to 50 µl of medium (without serum and antibiotics) or to PBS (see *chapter 3.2 for optimum amounts*).
   Gently mix the solution by pipetting up and down once.

   Solution B: Add 1.0 – 6.0 µl AppliFect to 50 µl of medium (without serum and antibiotics) or to PBS. (The optimum ratio of Lipid:nucleic acid may be 2 – 7 :1)
   Gently mix the solution by pipetting up and down once.

   For optimum ratio of the amount of solutions, please see chapter *3* and *4*.

5. Combine solutions A and B.
   Note the following order: Add DNA/RNA solution to the AppliFect solution.
   Do not shake, mix or vortex since shear force can damage the complex of DNA/RNA with lipids!

   Incubate 15-20 minutes at room temp.

6. Add the DNA/RNA:lipid complex to the cells immediately after the incubation period, mix very gently by swirling the cell culture vessel and incubate at 37° C in a CO_2 incubator.

   (In the case of extremely sensitive cells, the transfection solution is removed after 3 – 6 h and replaced with fresh complete medium).

7. Perform reporter gen assay after 24 – 72 h. (The incubation time largely depends on cell type and promoter activity.)

2.2 Standard protocol 2
Transfection of cells grown in suspension

1. In 1 ml of complete medium seed 0.4 – 1.6·10^5 cells on a 12-well culture dish.

   Optimum cell densities depend on the cell type and cell size.

2. Adjust DNA/RNA solutions as well as AppliFect to room temperature. Gently swirl individual solutions.

3. Prepare the following solutions.

   Use polypropylene plastics preferentially, otherwise use glass or polyethylene tubes.

   Always pipette medium first in order to prevent direct contact of AppliFect and DNA/RNA solutions to the surface of the vials.

   Solution A: Add 0.5 – 1.5 µg DNA/RNA to 50 µl of medium (without serum and antibiotics) or to PBS.
   Gently mix the solution by pipetting up and down once.

   Solution B: Add 1.0 – 6.0 µl AppliFect to 50 µl of medium (without serum and antibiotics) or to PBS.
   Gently mix the solution by pipetting up and down once.

   For optimum ratio of the amount of solutions, please see chapter *3* and *4*.

4. Combine solutions A and B.
   Note the following order: Add DNA/RNA solution to the AppliFect solution.
   Do not shake, mix or vortex since shear force can damage the complex of DNA/RNA:lipids!

   Incubate 15-20 minutes at room temp.

5. After the incubation time add the DNA/RNA:lipid complex drop by drop to the cell suspension.
   Mix very gently by rotating the cell culture vessel and incubate at 37°C in a CO_2 incubator.

   (In the case of extremely sensitive cells, the transfection solution is removed after 3 – 6 h and replaced with fresh complete medium).

8. Perform reporter gen assay after 24 – 72 h. (The incubation time largely depends on cell type and promoter activity.)

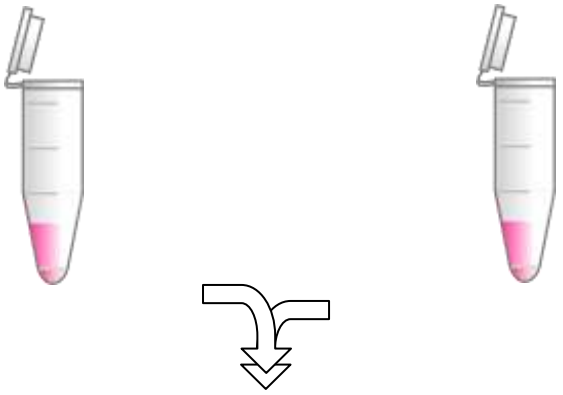
2.3 Short protocol Transfection

1. Seed 1–4 · 10^5 Cells

2. Incubate 18 – 24 h

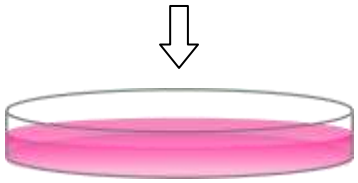
3.
Solution A
50 µl medium (without serum and antibiotics),
+ 0.5 – 1.5 µg DNA or RNA

Solution B
50 µl medium (without serum and antibiotics),
+ 1 – 6 µl AppliFect



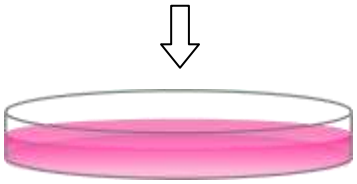
4.
Solution A + solution B
15 – 20 min @ RT

5.
DNA/RNA:Lipid complex
3 – 6 h



6. (optional)
Add fresh complete medium

7.
24 -72 h



8.
Reporter gen assay

PanReac AppliChem ITW Reagents

Optimizing Transfection Efficiency

Key Parameter for Optimization

3.1 Ratio of DNA/RNA:lipid complex

The most important optimization factor is the ratio of AppliFect to DNA/RNA. An excess of positively charged DNA/RNA:lipid complex is necessary for successful transfection. The optimal ratio depends on the particular cell line.

Table 1: Optimal AppliFect:DNA ratios for the 96-well format for different cell lines and DNA samples.

Cell line	Amount of DNA per welll		
	0.1 µg	0.2 µg	0.3 µg
293T	5:1	5:1	5:1
BHK	6:1	5:1	3:1
CaCo-2	7:1	7:1	7:1
CHO	6:1	6:1	5:1
COS-7	6:1	5:1	3:1
CV-1	6:1	6:1	6:1
HeLa	7:1	3:1	3:1
Hep G3	7:1	6:1	6:1
J774	4:1	3:1	3:1
MDCK	7:1	7:1	7:1
NIH 3T3	5:1	3:1	3:1
Vero	6:1	6:1	6:1

3.2 Total amount of DNA/RNA:lipid complex

In addition, the amount of the DNA/RNA:lipid complex influences the efficiency of transfections. However, an excess of lipid can lead to cell lysis.
The optimal ratio of AppliFect-DNA/RNA and total amount of the complex varies with the concentration of cells. Once the ideal ratio is established, the cell number and incubation time prior to transfections should be kept constant. This ensures reproducible results.

3.3 Number of cells

Please, note *chapter 1*.

3.4 Serum effects

Transfections using AppliFect can be performed in the absence of serum, at reduced serum concentrations (e.g. with 5% serum and OptiMEM, Invitrogen), or in complete medium containing 10% of serum. Cells transfected using AppliFect typically give best results with media containing serum. Individual cell lines may exhibit different behavior. In addition, the optimum ratio of AppliFect to DNA / RNA and the amount of the DNA / RNA-lipid complex may vary at different serum concentrations.

Important note: During the formation of lipid:DNA/RNA complex, no serum is allowed!
Serum will inhibit the formation of the complex. However, after the complex is formed the presence of serum will not interfere with transfection efficiency.

3.5 More Parameters for Optimization

The following parameters may lead to improved transfection efficiency. Change only one parameter at a time.

Incubation time

Cells may be incubated with the transfection complex (i.e. the lipid:DNA/RNA complex) over a wide range of periods (3 – 72 h). The optimum period for transfection depends largely on the cell type.

Period until reporter gen assay

Cells are tested for reporter gen activity 24 – 72 h after transfection. Cell type, promoter activity, and toxicity of the expressed product will influence the optimum incubation time.

Using PBS instead of serum-free medium during complex formation

In many experiments we found that using PBS during the formation of lipid:DNA/RNA complexes is preferable over the use of serum-free medium. More reproducible and in some cases, higher transfection rates have been achieved using PBS.

PBS composition:
10X PBS stock solution

40 g	NaCl
1 g	KCl
1 g	KH_2PO_4
5.75 g	$Na_2HPO_4 \cdot 2\ H_2O$

Dissolve the salts in 500 ml water
Autoclave 35 minutes at 121°C.

1X PBS:
In a volumetric flask dilute 100 ml of 10X PBS with 900 ml of water.
Autoclave 35 minutes at 121°C.

Adding transfection complex to freshly seeded cells

In some cases, a substantial increase in efficiency can be achieved if transfection of adherent cells is done shortly (within 1 hour) after they were seeded in appropriate culture vessels. In addition, the time for the protocol can be shortened this way by 24 hours.

3.6 General protocol for optimization

Use reporter gene plasmids such as pCMVßGal, pND2Luc, pEGFP.

1. Vary the amount of AppliFect: for example use 2 µl, 4 µl, 6 µl, 8 µl, 10 µl, 12 µl etc.)
   Keep the number of cells and amount of DNA (or RNA) constant in the tests.

2. Vary the amount of DNA (or RNA): for example use 1 µg, 1.5 µg, 2 µg, 2.5 µg, 3 µg
   Adjust the amount of AppliFect accordingly. Keep the number of cells constant in the tests.

3. Repeat step 1 and 2, but use media without serum or with reduced serum concentration.

4. Repeat step 1 and 2 with different cell numbers at the start of the experiment.

3.7 Protocol for optimization, using 12-well plates

1. Seed in a 12-well plate 1–4 $\cdot 10^5$ cells per well. Use 1 ml of appropriate complete growth medium.

   Optimum amounts of cells depend on cell type. Keep the same conditions between the experiments after the best values were established.

2. Incubate cells at 37°C in a CO_2 incubator until cells seem 90 – 100% confluent.

   (Also see chapter *1 and 4*).

3. Adjust DNA/RNA solutions as well as AppliFect to room temperature. Gently swirl individual solutions.

4. Give 50 µl of serum-free and antibiotic-free medium or PBS to each position of a 96-well plate. Then add the following amounts of DNA (or RNA):

   0.5 µg DNA/RNA to A1-A4

   1.0 µg DNA/RNA to B1-B4

   1.5 µg DNA/RNA to C1-C4

   Gently mix the solutions by pipetting up and down once.

5. Give 50 µl of serum-free and antibiotic-free medium or PBS to each position given below of the 96-well plate. Then add:

   1 µl, 2 µl, 4 µl, 6 µl AppliFect to D1-D4

   2 µl, 4 µl, 8 µl, 12 µl AppliFect to E1-E4

   4 µl, 8 µl, 12 µl, 16 µl AppliFect to F1-F4

   Gently mix the solutions by pipetting up and down once.

   Note the following order: Add DNA/RNA solution to the AppliFect solution.

   Combine the solutions according to the following scheme: A1+D1, A2+D2 etc., B1+E1, B2+E2 etc., C1+F1, C2+F2 etc.) **Do not shake, mix or vortex since shear force can damage the complex of DNA/RNA:lipids!**

   Incubate 15 – 20 minutes at room temp.

6. Add the DNA/RNA:lipid complex to the cells immediately after the incubation period, mix very gently by rotating the cell culture vessel and incubate at 37°C in a CO_2 incubator.
   (In the case of extremely sensitive cells, the transfection solution is removed after 3 – 6 h and replaced with fresh complete medium).

7. Perform reporter gen assay after 24 – 72 h. (The incubation time largely depends on cell type and promoter activity.)

   If the results are satisfactory, adjust the volumes. Please see the next *chapter 3.8 Up- und Downscale* for things that need to be considered.

3.8 Up- and Downscale

The following table provides amounts of the reagent for transfection in different formats (number in brackets give recommended starting values).
Please note:
In our experience the optimal ratio of AppliFect [µl] : DNA/RNA [µg] is between 2:1 and 7:1 (see Tab. 1, *chapter 3.1*).
With significant size differences the optimum ratios differ (due to the absorption to the surface of the culture vessel of different sizes)

Tabelle 2: Amounts and volumes for transfections with different culture sizes

Cell numbers and DNA/RNA amounts in parentheses are proven good starting values for optimization

Diameter [mm] culture disc/plate	**7 (96-Well)**	**16 (24-Well)**	**22 (12-Well)**	**35 (6-Well)**	**60**	**100**
Growth area [cm x cm]	0.31	1.9	3.7	9	22	60
Proportional factor	0.03	0.2	0.4	1.0	2.5	6.7
Cell number seeded for adherent cells* [x 10^5] (1 day prior to transfection)	0.10 – 0.60 (0.30)	0.4 – 2.0 (1.0)	1.0 – 4.0 (2.0)	2.5 – 10.0 (5.0)	6.0 – 24.0 (12.0)	15.0 – 60.0 (25.0)
Cell number seeded for cells grown in suspension* [x 10^5] (at the day of transfection)	0.04 – 0.24 (0.12)	0.16 – 0.8 (0.40)	0.4 – 1.6 (0.8)	1.0 – 4.0 (2.0)	2.4 – 9.6 (4.8)	6.0 – 24.0 (10.0)
Volume of cell suspension [ml]	0.15	0.5	1.0	2.0	4.5	12.0
Amount of DNA/RNA [µg]	0.05 – 0.3 (0.1)	0.15 – 1.0 (0.5)	0.3 – 2.0 (1.0)	0.4 – 5.0 (2.0)	1.0 – 12.0 (6.0)	2.0 – 34.0 (14.0)
AppliFect [µl]	0.2 – 4.0 (0.6)	0.5 – 7.0 (2.0)	1.0 – 15.0 (3.0)	2.0 – 35.0 (6.0)	4.0 – 90.0 (18.0)	10 - 250 (42.0)
Volume for dilution of DNA/RNA [µl]	15 - 30	30	50	100	300	700
Volume for dilution of AppliFect[µl]	10 - 50	10 - 50	50	100	300	700
Total volume [ml]	**0.175 – 0.23**	**0.54 – 0.58**	**1.1**	**2.2**	**5.1**	**13.4**

* Optimal cell concentrations depend on cell type and size.

Troubleshooting guide

1. Avoid direct contact of undiluted AppliFect solution or undiluted DNA/RNA solution with cell culture vessel or well (such as 96-well plates).
   ⇨ Pipette medium or PBS first. Then add AppliFect and DNA/RNA solution respectively.
2. Combine diluted solutions of DNA/RNA and AppliFect immediately (within 5 minutes after dilution). Add DNA/RNA solution to the AppliFect solution.
3. Overexpression may cause reduction of cell proliferation or cytotoxic effects.

   ⇨ Use higher cell numbers or reduce the amount of transfection complex solution to compensate for such effects.
4. Do not use antibiotics during transfection. This may cause cell death and will result in low transfection efficiency.
5. In the case of sensitive cells, the transfection solution should be removed after 3 – 6 hours, replace with fresh complete medium.