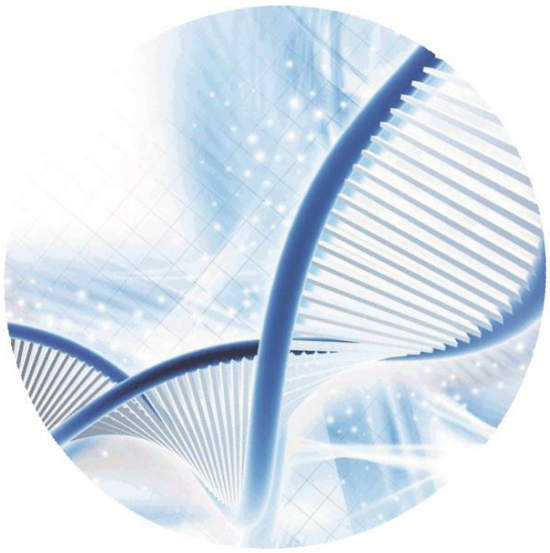

Transfection reagent

DreamFect Gold™

Tee Technology (Triggered Endosomal Escape)
DNA Delivery

Protocol

We Bring The World Of Biotechnology To You
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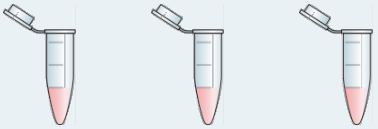
The art of delivery systems

DreamFect Gold Quick Protocol

To find the ideal conditions, DreamFect™ Gold must be tested at ratios of **2 µL/µg**, **3 µL/µg** and **4 µL/µg** (µL of DreamFect Gold / µg of DNA). For the DNA quantity, we suggest **0.25 µg** per well in 96-well, **0.5 µg** per well in 24-well and **1 µg** per well in 6-well.

1 **Seed cells to be at 70% confluent the day of transfection***

2 **Prepare 3 identical tubes of DNA**

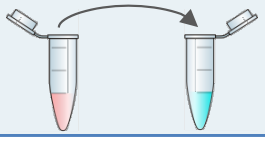


96 well plate	24 well plate	6 well plate
0.25µg in 25µL of serum-free medium or buffer* X 3	0.5µg in 50µL of serum-free medium or buffer* x 3	1µg in 100µL of serum-free medium or buffer* x 3

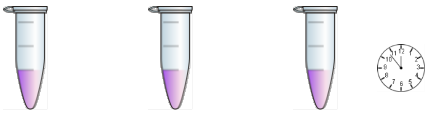
3 **Prepare 3 tubes of DreamFect™ Gold (with 3 different amounts of reagent)**

96 well plate	24 well plate	6 well plate
0.5µL/0.75µL/1µL in 25µL of serum-free medium or buffer*	1µL/1.5µL/2µL in 50µL of serum-free medium or buffer*	2µL/3µL/4µL in 100µL of serum-free medium or buffer*

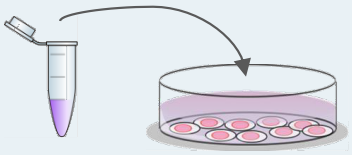
4 **Mix each tube of DNA (step 2) to each tube of DreamFect™ Gold (step 3)**



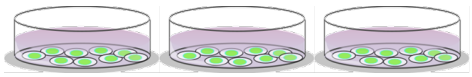
5 **Incubate 15 to 20 min at room temperature**



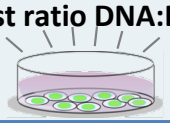
6 **Distribute each mix dropwise onto the cells to insure uniform distribution**



7 **Incubate cells for 24 to 72h at 37°C until evaluation of transgene expression***



8 **Choose the best ratio DNA:DreamFect™ Gold**



These conditions might require some further optimizations depending on your cells, DNA, RNA, etc.

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ For cell lines, seed the cells 24h before transfection in a 96-well plate, 24-well plate or 6-well plate in respectively 150 µL, 400 µL and 2 mL of complete culture medium.
- ✓ Allow reagents to reach RT and gently vortex them before forming complexes.
- ✓ Medium or buffer without serum & supplement must be used for the DNA/DreamFect Gold complexes preparation. Culture medium such as MEM, DMEM or OptiMEM or buffers such as HBS or PBS are recommended. In contrast, we do not recommend RPMI for preparing the complexes.
- ✓ Dilute the reagent with deionized water for doses less than 1µL.
- ✓ For some cells, 24h post-transfection replace the medium with fresh pre-warm medium or just add fresh growth culture medium to the cells. In the case of cells very sensitive to transfection, the medium can be replaced after 3-4h.

DreamFect Gold Reagent | Specifications

Package content	DG80500: 500µL of DreamFect Gold DG81000: 1mL of DreamFect Gold DG85000: 5 x 1mL of DreamFect Gold
Shipping conditions	Room Temperature
Storage conditions	Store the DreamFect Gold transfection reagent at -20°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product Descriptions	DreamFect Gold is a lipopolyamine formulation specifically designed to achieve high transfection efficiency in a wide variety of cell lines and primary cells
Important notice	For research use only. Not for use in diagnostic procedures

Protocol | DNA or shRNA vectors transfection in adherent cells

1. Cells Preparation

It is recommended to seed or plate the cells the day prior transfection. The suitable cell density will depend on the growth rate and the conditions of the cells. Cells should not be less than 60 % confluent (percentage of growth surface covered with cells) at the time of transfection (see Table 1). The correct choice of optimal plating density also depends on the planned time between transfection and transgene analysis: for a large interval, we recommend a lower density and for a short interval a higher density may be advantageous.

Tissue Culture Dish	Adherent Cell Number
96 well	0.05 – 0.2 x 10^5
24 well	0.5 – 1 x 10^5
6 well	2 – 5 x 10^5

Table 1: Recommended cell number depending on the culture well format

2. DNA/DreamFect Gold complexes preparation

a. *DreamFect Gold*: Vortex the reagent and dilute the indicated quantity of DreamFect Gold in 25 to 100 µL of culture medium without serum and supplement (see Table 2).

b. *DNA*: Dilute the indicated quantity of DNA (see Table 2) in 25 to 100 µL of culture medium without serum and supplement.

c. Add DNA solution to DreamFect Gold solution, mix gently by carefully pipetting up and down and incubate the mixture at room temperature for 15-20min. Do not vortex or centrifuge.

3. Transfection

a. Add the complexes onto cells drop by drop and gently rock the plate to ensure a uniform distribution.

b. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of transgene expression.

Tissue Culture Dish	DNA Quantity (µg)	DreamFect Gold Volume (µL)	Dilution Volume (µL)	Transfection Volume
96 well	0.25	0.75	2 x 25	200 µL
24 well	0.5	1.5	2 x 50	500 µL
6 well	1	3	2 x 100	2 mL

Table 2: Recommended DNA amount, DreamFect Gold volume and transfection conditions

NOTE: In case of cells very sensitive to transfection, the medium can be changed after 3-4 h or 24 h incubation with fresh medium.

IMPORTANT OBSERVATION FOR PROTEIN PRODUCTION OVER 24H

In case of protein production experiment over 24h, we recommend using two times more amounts of DNA per well to yield maximal levels of protein.

Protocol | DNA or shRNA vectors transfection in suspension cells

1. Cell Preparation

The day before transfection split the cells at a density of 2 to 5 x 10^5 cells / mL, so they are in excellent condition on the day of transfection. Incubate overnight in complete culture medium.

2. DNA/DreamFect Gold complexes preparation

a. *DreamFect Gold*: Vortex the reagent and dilute the indicated quantity of DreamFect Gold in 25 to 100 µL of culture medium without serum and supplement (see Table 3).

b. *DNA*: Dilute the indicated quantity of DNA (see Table 3) in 25 to 100 µL of culture medium without serum and supplement.

c. Add the DNA solution to the DreamFect Gold solution, mix gently by carefully pipetting up and down and incubate the mixture at room temperature for 15-20min. Do not vortex or centrifuge.

Tissue Culture Dish	Suspension Cell Number	DNA Quantity (µg)	DreamFect Gold Volume (µL)	Dilution Volume (µL)	Transfection Volume
96 well	0.5 – 1 x 10^5	0.25	0.5 - 1	2 x 25	100 µL
24 well	2 - 4 x 10^5	1	2 - 4	2 x 50	250 µL
6 well	10 - 15 x 10^5	3	6 - 12	2 x 100	1 mL

Table 3: Suggested transfection conditions for suspension cells

3. Transfection

a. While the complexes are incubating, prepare your cells in serum-free medium (or serum-containing medium) and transfer the appropriate volume to the culture dish according to Table 3. In 24-well plates for instance plate 2x10^5 suspension cells just before transfection in 250 µL of serum free medium. Generally, serum-free condition leads to higher transfection efficiency.

b. Next, add the complexes directly onto the cells dropwise and all over the well. Important: gently mix complexes with the cells by pipetting the culture medium up and down (3-4 times to disrupt potential cell clumps and to ensure contact of the complexes with cells).

c. Incubate 3 to 6 h (4h is commonly used) in serum-free medium at 37°C under 5% CO2.

d. If transfection is performed in serum free medium, add serum to adjust its concentration.

e. Incubate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of transgene expression. Depending on the cell type and promoter activity, the assay for the reporter gene can be performed 24 to 72h following transfection.

NOTES:

- For some cells, 24 h post-transfection replace the old media with fresh media or just add fresh growth culture medium to the cells.
- In case of cells very sensitive to transfection, the medium can be changed after 3-4 h or 24 h incubation with fresh medium.

IMPORTANT OBSERVATIONS

- Transfections are optimum when performed in the absence of serum. However, transfection can also be achieved directly in the presence of serum.

- The key feature is to promote as much as you can the contact between the cells and the transfection complexes. We suggest:

    - **Option 1** - concentrate your cells: When the complexes are forming prepare your cells. Spin down the cells, resuspend them at 10×10^{6} cells / mL in medium (serum free) and transfer the appropriate cell number to your well according to Table 3. Thereafter, mix the complexes with the cells, incubate 15 min and complete the culture medium as indicated in Table 3.
    - **Option 2** - promote contact by centrifugation: Centrifuge the plate after having mixed the cells with the transfection complexes for 2-3 min at around 1000-1200 rpm.

- GeneBlaster™ Topaz can be used to boost the gene expression level in some cell lines.

Protocol | DNA Co-transfection

For co-transfection of several plasmids DNA, mix the same amount of each plasmid and transfect as described above. For example, if you have two DNA plasmids, mix 0.25 µg of each plasmid, complex the 0.5 µg of DNA with 1.5 µL of DreamFect Gold.

Option for co-transfection

Transfections can be realized sequentially instead of simultaneously. So, cells can be transfected with one plasmid DNA first and 4h to 24h later can be transfected with the other plasmid DNA. Follow the procedure as detailed above for DNA transfection. A medium changed can be also performed between the two transfections.

Protocol | Stable transfection

The same protocol can be used to produce stably transduced cells except that 48h post-transfection, cells are transferred to fresh medium containing the appropriate antibiotics for selection. It is important to wait at least 48h before exposing the transduced cells to selection media. For suspension cells, we suggest exposing the cells to selection media at least 72h post-transfection.

1. Cell Preparation siRNA/DreamFect Gold complexes preparation

The day prior transfection, prepare the cells as described in Table 1. Generally, siRNA transfection requires lower cell density than DNA transfection. The correct choice of optimal density depends on the planned time between transfection and gene knockdown analysis: for a large interval, we recommend a lower density and for a short interval a higher density may be advantageous.

2. siRNA/DreamFect Gold complexes preparation

The siRNA and DreamFect Gold solutions should have an ambient temperature and be gently vortexed prior to use.

a. *siRNA solution*. Dilute the siRNA stock solution (for instance 1µM stock solution) in 50 or 100 µL (see Table 4) of culture medium without serum and antibiotics.

Culture vessel	96-well		24-well		6-well	
Dilution serum-free medium	50µL		50 µL		100 µL	
*Amount of siRNA (1 µM stock)**						
Final siRNA concentration	(µL)	(ng)	(µL)	(ng)	(µL)	(ng)
10 nM	2	27	5	67.5	20	270
20nM	4	54	10	135	40	540
50 nM	10	135	25	337.5	100	1350

* ng of siRNA was calculated on the basis of a MW = 13 500

Table 4: Suggested dilution procedure and amount of siRNA to test

b. *DreamFect Gold preparation*. Dilute 0.5 to 5 µL of DreamFect Gold in 50 or 100 µL of culture medium without serum and antibiotics (see Table 5 for corresponding volumes of DreamFect Gold).

Culture vessel	96-well	24-well	6-well
Dilution serum-free medium	50 µL	50 µL	100 µL
Final transfection Volume	200 µL	500 µL	2 mL
Amount of DreamFect Gold (µL)			
Final siRNA concentration			
10 nM	0.15	0.5	2
20nM	0.3	1	4
≥ 50 nM	0.5	2	8

Table 5: Recommended amount of DreamFect Gold per nM of siRNA used

c. Add the siRNA solution onto the DreamFect Gold reagent. Mix gently by carefully pipetting up and down and incubate the mixture for 15 min at room temperature. Do not vortex or centrifuge!

3. Transfection.

a. Add the complexes onto the cells and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture.

b. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of gene knockdown analysis. We recommend 24h for RNA analysis and 48h to 72h for protein knockdown analyses.

NOTES:

- Depending on the siRNA amount, the gene targeted and the cell type, assays can be monitored 24 to 96h post-transfection
- For some cells, 24h post-transfection replace the old media with fresh media or just add fresh growth culture medium to the cells.
- If cells are very sensitive to transfection, the medium can be changed after 3-4h or 24h incubation.

IMPORTANT OBSERVATIONS

- Ensure to avoid the presence of serum when preparing the transfection reagent/siRNA complexes. Use a medium well pH (some old medium can turn pink or purple instead of being orange or red) which could influence complexes formation and siRNA stability.
- Avoid incubating your diluted siRNA too long in your serum-free medium; prepare first your transfection reagent, dilute your siRNA and quickly transfer the diluted siRNA into the DreamFect™ Gold tube.
- Start with 50 nM siRNA concentration and test four amounts of DreamFect Gold™.
- The gene silencing is highly dependent on your protein half-life and consequently it will be good to analyze your protein expression by western at 48h, 72h and 96h.
- Treating your cells twice with 25nM siRNA instead of once with 50nM can enhance significantly siRNA effects. Basically, on day one, incubate your cells with 25nm siRNA and DreamFect™ Gold. On day two, change your medium and repeat the treatment with 25nm siRNA and DreamFect™ Gold.

Optimization Protocol

We recommend optimizing the transfection protocol for each combination of plasmid and cell line used in order to get the best out of DreamFect™ Gold. Several parameters can be optimized:

- Ratio of DreamFect™ Gold to nucleic acid
- Dose of nucleic acid used
- Cell type and cell density
- Culture medium composition (+/- serum) and reagent / nucleic acid complex medium
- Incubation time

We recommend that you optimize one parameter at a time while keeping the other parameters (cell number, incubation time etc.) constant. The two most critical variables are the ratio of DreamFect™ Gold reagent to DNA (or siRNA) and the quantity of DNA (orsiRNA concentration).

1. DreamFect™ Gold / DNA ratio

This is an important optimization parameter. DreamFect™ Gold has to be used in slight excess compare to DNA but the optimal ratio will depend on the cell line and the vessel used. It is particularly true for 96 well plates because of adsorption processes. For optimization, first maintain a fixed quantity of DNA (according to the size of your culture dish or cell number) and then vary the amount of DreamFect™ Gold reagent over the suggested range in the table 6. You can test ratios from 1 to 6 µl of DreamFect™ Gold reagent per 1 µg DNA.

Tissue Culture Dish	DNA Quantity (µg)	DreamFect Gold Volume (µL)	DreamFect Gold Volume (µL) proposed interval
96 well	0.1	0.1 – 0.6	0.1 –0.2 -0.3 -0.4 -0.5 – 0.6
24 well	0.5	0.5 – 3	0.5 – 1- 1.5 – 2– 2.5 - 3
6 well	2	2 – 12	2 - 4 – 6 - 8 – 12

Table 6: Suggested range of DreamFect™ Gold for optimization

2. DreamFect™ Gold / siRNA ratio

Start by optimizing the ratio of DreamFect Gold / siRNA. To this end, use a fixed amount of siRNA and vary the amount of DreamFect™ Gold as detailed in the Table 7. Diluted DreamFect™ Gold solution in deionized water for volumes less than 1 µL must be freshly prepared.

Tissue culture dish	96 well	24 well	6 well
Dilution serum-free medium	50 µL	50 µL	100 µL
Final transfection volume	200µL	500µL	2mL
Amount of DreamFect Gold			
Final siRNA Concentration			
25nM	0.15 – 0.3 – 0.45 – 0.6µL	0.5 – 1 – 1.5 – 2µL	2 – 4 – 6 – 8µL
50nM	0.25 – 0.5 – 0.75 – 1µL	1 – 2 – 3 – 4µL	4 – 8 – 12 – 16µL

Table 7: Recommended amount of DreamFect™ Gold per nM of siRNA used

3. Quantity of DNA or siRNA

To achieve the optimum transfection efficiency, the amount of nucleic acid used (DNA or siRNA) can be optimized. Keep the number of cells and the incubation time constant and adjust the quantity of nucleic acid while maintaining a fixed ratio of DreamFect™ Gold reagent to DNA or siRNA (Table 8).

Tissue Culture Dish	DNA Quantity (µg)	Transfection Volume
96 well	0.1 – 0.5	200 µL
24 well	0.25 – 2	500 µL
6 well	2 – 10	2 mL

Table 8: Suggested range of DNA amounts for optimization

Thereafter, culture medium compositions, cell number, incubation times can also be optimized.

4. DreamFect Gold / Nucleic acid complex medium

Several tests demonstrated that the use of PBS to prepare complexes instead of serum- and antibiotic-free medium leads to more reproducible transfections and in some cases higher efficiency, particularly with lower volumes of transfection reagent. PBS composition: 137mM NaCl, 2.7mM KCl, 1.5mM KH_2PO_4 and 6.5mM Na_2HPO_4 x 2 H_2O; pH7.4.

5. Cell number

The cell proliferating rate is also a critical parameter and the optimal confluency has to be adjusted according to the cells used. Thus, the next step is to use the optimized parameters obtained previously and vary the cell number to be assayed.

NOTE: The addition of the transfection complex directly to fresh seeded cells can result in a considerable increase of transfection efficiency.

6. Effect of serum /Transfection volume

Almost all cell lines transfected with DreamFect™ Gold showed superior results if serum is present during the transfection. Some cell lines may behave differently and transfection efficiency can be increased without serum or under reduced serum condition. **Remember that presence of serum during complex formation is strictly prohibited, as the serum will inhibit their formation.** Transfection efficiency is attained when the initial 3-4 h of incubation is done. Consequently, the cells may be kept in serum-free or reduced serum conditions during the first 4 h of transfection. If you use serum-free medium, replace it by a culture medium containing serum or just add serum to the wells according to your standard culture condition after this period. To increase the efficiency of transfection you can reduce the transfection volume.

7. Incubation time

The optimal time range between transfection and assay for gene activity varies with cell line, promoter activity, expression product, etc. The transfection efficiency can be monitored after 24 - 96 h by analyzing the gene product.

Additional products

- **DreamFect Stem** dedicated to stem cells transfection
- **PolyMag Neo** – magnetic beads-based transfection reagent dedicated to hard-to-transfect cells
- **pVectOZ Transfection plasmids (CAT, GFP, LacZ, Luciferase, SEAP)** - Positive controls and optimization of all transfection experiments

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