DreamFect™ Gold
INSTRUCTION MANUAL

OZ BIOSCIENCES
The art of delivery systems

DreamFect™ Gold

Instruction Manual

DreamFect™ Gold

The newest generation of outstanding transfection reagent that achieves high transfection efficiency combines with superior transgene expression level

List of DreamFect™ Gold Kits

Catalog Number	Description	Volume (µL)	Size (number of transfection / µg of DNA)
DG80500	DreamFect Gold ™	500	125 to 500
DG81000	DreamFect Gold ™	1000	250 to 1000
DG85000	DreamFect Gold ™	5 X 1000	1250 to 5000

Use the content of the table above to determine the appropriate catalog number for your needs. You can order these products by contacting us (telephone, fax, mail, e-mail) or directly through our website. For all other supplementary information, do not hesitate to contact our dedicated technical support: tech@ozbiosciences.com.

OZ BIOSCIENCES
Parc Scientifique et Technologique de Luminy
B.P. 13
13273 Marseille Cedex 9
France
Tel: +33 (0) 4.91.82.81.72
Fax: +33 (0) 4.91.82.81.70
E-mail: contact@ozbiosciences.com
Web Site: www.ozbiosciences.com

OZ BIOSCIENCES
The art of delivery systems

Table of Contents

1. Technology **2**
- 1.1. Description 2
- 1.2. Kit Contents 2

2. Applications **3**
- 2.1. Application Areas 3
- 2.2. Cell Types 3
- 2.3. DreamFect™ Gold and Magnetofection™ 3

3. General Protocols **3-10**
- 3.1. General Considerations 3
- 3.2. Cell Preparation 4
- 3.3. Rapid Protocol – Adherent Cells 4-5
- 3.4. Suspension Cells Protocol 5-6
- 3.5. siRNA Transfection Protocol 6-7
- 3.6. Co-Transfection siRNA-DNA Protocol 7-8
- 3.7. Optimization Protocol 8-10

4. Appendix **10-11**
- 4.1. Quality Controls 10
- 4.2. Troubleshooting 10-11

5. Related Products **12**

6. Purchaser Notification **13**

1. Technology

1.1. Description

Congratulations on your purchase of the ***DreamFect™ Gold*** transfection reagent!
DreamFect™ Gold is the newest transfection reagent based on the **Tee-Technology ("Triggered Endosomal Escape")** which combines and exploits the properties of cationic lipids and polymers to achieve an extremely efficient DNA delivery into cells. Because the level of transgene expression matters as well as the transfection efficiency, OZ Biosciences has developed the next powerful generation of lipopolyamines with improved cytoplasmic release process and better biodegradability, ***DreamFect™ Gold***. In this way, high transfection efficiency combines with superior transgene expression level are achieved. DreamFect™ Gold can be used with all types of nucleic acids and allows their delivery in a wide variety of cell lines or primary cells.

Principal **DreamFect™ Gold** advantages:

1. Highly efficient
2. Achieve greater transgene expression level than any other reagents
3. Complete Biodegradability
4. Universal (primary cells and cell lines)
5. Multipurpose (various types of nucleic acid)
6. Simple, ready-to-use & rapid
7. Serum compatible
8. Ideal for High Throughput Screening

1.2. Kit Contents

OZ Biosciences offers three sizes of DreamFect™ Gold reagents.

- One tube containing 500 µL of DreamFect™ Gold good for 125 to 500 transfections with 1 µg of DNA
- One tube containing 1 mL of DreamFect™ Gold good for 250 to 1000 transfections with 1 µg of DNA
- 5 tubes containing 1 mL of DreamFect™ Gold good for 1250 to 5000 transfections with 1 µg of DNA

Stability and Storage
Storage: Upon reception and for long-term use, store the reagent at -20°C.
DreamFect™ Gold kits are very stable for at least one year at room temperature, +4°C or -20°C. The storage at -20°C minimizes the size of liposomes and thus leads to higher efficiency. Nonetheless, the reagent can also be stored at +4°C. The numbers of freeze and thaw cycles do not affect the efficiency of the reagent.

Shipping condition: Room Temperature.

2. Applications

2.1. Application Areas

DreamFect™ Gold has been developed for very efficient transfections of various types of nucleic acids such as **DNA, mRNA, siRNA or oligonucleotides** in a wide variety of immortalized and primary cells. This transfection reagent is serum compatible and can be used for transient and stable transfection. This product is very stable, ready-to-use and intended for research purpose only.

2.2. Cell Types

DreamFect™ Gold transfection reagent is suitable for numerous cell types. It has been successfully tested on a variety of immortalized cell lines as well as some primary cells. An updated list of transfected cells is available on OZ Biosciences website: www.ozbiosciences.com. You can also submit your data to tech@ozbiosciences.com so we can update this list and give you all the support you need.

2.3. DreamFect™ Gold and Magnetofection™

DreamFect™ Gold reagent can also be combined with our Magnetofection™ transfection technology (CombiMag). This approach is particularly useful for very difficult to transfect cells such as certain primary cells. The combination of the two technologies will allow you to use very small amounts of DNA and increase the overall efficiency of your transfections. For further information concerning the Magnetofection™ transfection technology, see our website: www.ozbiosciences.com.

3. General Protocols

3.1. General Considerations

The instructions given below represent sample protocols that were applied successfully with a variety of cell lines. Optimal conditions may vary depending on the nucleic acid, cell types, size of cell culture dishes and presence or absence of serum. Therefore, the amounts and ratio of the individual components (DNA and DreamFect™ Gold) may have to be adjusted to achieve best results since each cell line has a particular optimal transfection reagent / nucleic acids ratio. As a result, we suggest you to optimize the various transfection parameters as described in section **3.7)** Optimization Protocol. The following recommendations can be used as guidelines to quickly achieve very good transfection and high transgene expression level. As a starting point, we recommend to use **4 µL of DreamFect™ Gold / 1 µg of DNA.** DreamFect™ Gold can be used in the presence or in the absence of serum. You can use your routine culture medium for the transfection, except during preparation of the DreamFect™ Gold / DNA complexes (see **3.3** below).

- **Cells** should be healthy and assay during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) will considerably affect the transfection efficiency. The cell proliferating rate is a critical parameter and the optimal confluency has to be adjusted according to the cells used. We recommend using regularly passaged cells for transfection and avoid employing cells that have been cultured for too long (> 2 months). Generally, siRNA transfection requires lower cell density than DNA transfection.
- **Nucleic acids** should be as pure as possible. Endotoxins levels must be very low since they interfere with transfection efficiencies. Moreover, we suggest avoiding long incubation time of the DNA/RNA solution in buffers or serum free medium before the addition of DreamFect™ Gold reagent to circumvent any degradation or surface adsorption.
- **Antibiotics**. The exclusion of antibiotics from the media during transfection has been reported to enhance gene expression levels. We did not observe a significant effect of the presence or absence of antibiotics with the DreamFect™ Gold reagent and this effect is cell type dependent and usually small.
- **Materials**. We recommend using polypropylene tubes to prepare the DNA and transfection reagent solutions but glass or polystyrene tubes can also be used.

A protocol used for other transfection reagents should never be employed for DreamFect™ Gold and inversely. Each transfection reagent has its own molecular structure, biophysical properties and concentration, which have an important influence on their biological activity.

3.2. Cells Preparation

Adherent cells. 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 the suggested cell number in the 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. (See section 3.3 for procedure)

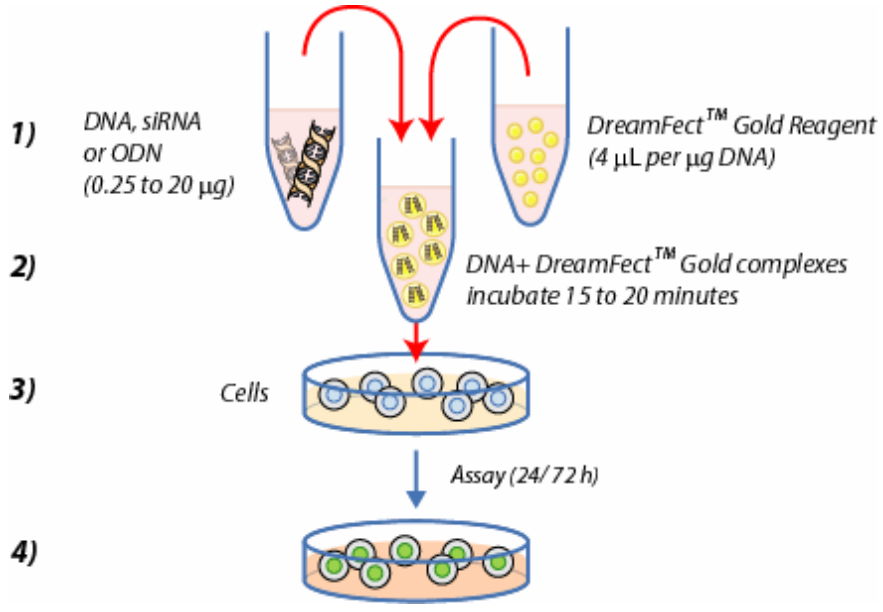
Suspension cells. For fast growing cells, split the cells the day before transfection at a density of 2 to 5 x 10^5 cells / ml, so they are in excellent condition on the day of transfection. (See section 3.4 for procedure)

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

Table 1: Cell number, DNA amount, DreamFect Gold volume and transfection conditions suggested.

Tissue Culture Dish	Adherent Cell Number	DNA Quantity (µg)	DreamFect Gold Volume (µL)	Dilution Volume (µL)	Transfection Volume
96 well	0.05 – 0.2 x 10^5	0.25	1	2 x 25	200 µL
24 well	0.5 – 1 x 10^5	1	4	2 x 50	500 µL
12 well	1 – 2 x 10^5	2	8	2 x 50	1 mL
6 well	2 – 5 x 10^5	3	12	2 x 100	2 mL
60 mm dish	5 – 10 x 10^5	6	24	2 x 150	4 mL
90 - 100 mm	10 – 30 x 10^5	12	48	2 x 250	8 mL
T-75 flask	20 – 50 x 10^5	20	80	2 x 350	10 mL

3.3. Rapid Protocol – Adherent Cells



The DNA and DreamFect™ Gold solutions should have an ambient temperature and be gently vortexed prior to use. The rapid protocol is as simple as follows: Use 4 µL of DreamFect™ Gold per µg of DNA. We suggest beginning with this ratio and optimize it, if required, by following section 3.7.

Important considerations before beginning transfection:

- DreamFect™ Gold can be stored at +4°C or -20°C. The storage at -20°C minimizes the size of liposomes and thus leads to higher efficiency. If transfections are performed routinely, the transfection reagent can be kept at 4°C for one week after thawing. Thereafter, the reagent should be stored back at -20°C.
- Do not use serum-containing media for this step!

- Prevent the DreamFect™ Gold and DNA stock solutions to come into contact with any plastic surface. First, add serum-free culture medium to the tube and then drop the DreamFect™ Gold and DNA stock solution directly into the medium. Contact of DreamFect™ Gold and DNA with the tube surface (plastic or glass) will result in materials lost by adsorption.

1) **DNA solution**. Dilute 0.25 to 20 μg of DNA in 25 to 350 µL of PBS* or culture medium without serum and antibiotics (see Table 1). (* for more information see section 3.7.4)

2) **DreamFect™ Gold solution**. Thaw the reagent at room temperature (the reagent can be warm in your hands for faster thawing). Dilute 1 to 80 µL of DreamFect™ Gold in 25 to 350 µL (see Table 1) of PBS* or culture medium without serum and antibiotics.

3) Add the DNA solution into the DreamFect™ Gold solution, mix gently by carefully pipetting up and down 2-3 times and incubate the mixture for 15-20 minutes at room temperature. Do not vortex or centrifuge!

    - **The diluted solutions should be combined within 5 minutes.**

4) Add the complexes (dropwise) to the cells growing in serum-containing culture medium and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture.

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

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

* DreamFect™ Gold / DNA complexes are prepared in medium without serum or PBS because serum interferes with vector assembly. Then, the serum free complexes cocktail is added to the cells that are covered with complete medium. Thus, the addition of the transfection cocktail results in the dilution of supplements such as serum, antibiotics or other additives of your standard culture medium. Although a medium change after transfection is not required for most cell types, it may be necessary for cells that are sensitive to serum/supplement concentration. Alternatively, the cells may be kept in serum-free medium during the first 4 hours of transfection and then a medium change can be performed.

3.4. Suspension Cells Protocol

The DNA and DreamFect™ Gold solutions should have an ambient temperature and be gently vortexed prior to use. For suspension cells, we highly recommend to test several amount of DNA with a transfection reagent/DNA ratio constant or several ratios with one amount of DNA according to section 3.7. The rapid protocol is as simple as follows: Use 3 µL of DreamFect™ Gold per µg of DNA.

1) 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) The day of transfection prepare the DreamFect™ Gold / DNA complexes as described above (section 3.3)

    - **For suspension cells, we suggest testing three DNA amounts while keeping the DNA/DreamFect Gold ratio constant (1/3).**

3) Thereafter, add the DNA solution into the DreamFect™ Gold solution, mix gently by carefully pipetting up and down 2-3 times and incubate the mixture for 15-20 minutes at room temperature. Do not vortex or centrifuge! Do not incubate the complexes more than 30 min.

4) 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 2. For instance, in 24-well plate, 2x10^5 suspension cells plated just before transfection in 250 µL of serum free medium. Generally, serum-free condition leads to higher transfection efficiency.

Table 2: Transfection conditions suggested for suspension cells.

Tissue Culture Dish	Suspension Cell Number	DNA Quantity (µg)	DreamFect Gold Volume (µL)	Transfection Volume
96 well	0.5 – 1 x 10^5	0.5	1.5	100 µL
24 well	2 - 4 x 10^5	2	6	250 µL
6 well	10 - 15 x 10^5	6	18	1 mL
60 mm dish	5 x 10^6	12	36	2.5 mL

5) 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). This step is important to ensure contact of the complexes with cells that have a tendency to clump. It is important to promote the contact of the complexes with cells during this mixing procedure. Gentle pipetting of the cells with the complexes disrupts these clumps that are preventing the complexes to get access to all cells and produces a single-cell suspension which will increase transfection efficiency.

6) Incubate 3 to 6 h (4 hours is commonly used) in serum-free medium at 37°C under 5% CO_2. If serum-containing medium is used, then proceed to step 8.

7) Add more culture medium containing 20 % serum (same volume than the transfection volume).

8) 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 72 hours following transfection.

   - **For some cells, 24 hours post-transfection replace the old media with fresh media or just add fresh growth culture medium to the cells. * (see remark in section 3.3.5)**

Important Observations:

A. Note that transfections are optimum when performed in the absence of serum. However, transfection can also be achieved directly in the presence of serum.

B. From our experiences, the key feature is to promote as much as you can the contact between the cells and the transfection complexes. Consequently, here a few additional proposition:

   - 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 2. Thereafter, mix the complexes with the cells, incubate 15 minutes and complete the culture medium as indicated in Table 2.

   - Option 2, promote contact by centrifugation: Centrifuge the plate after having mixed the cells with the transfection complexes for 2-3 minutes at around 1000-1200 rpm.

C. For some haematopoietic cells, such as Jurkat, ***GeneBlaster™ Topaz*** (catalog # GB20013) can be used to boost the gene expression level.

3.5. siRNA Transfection Protocol

1) The day prior transfection, prepare the cells as described in section 3.2. 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, be gently vortexed prior to use and be combined within 5 minutes.
   a. Dilute the siRNA stock solution (for instance 1µM) in 25 or 50 µL (see Table 3) of PBS or culture medium without serum and antibiotics. We advise starting with a final siRNA concentration of 50nM.
   b. Dilute the DreamFect Gold reagent in 25 or 50 µL (see table 4) of PBS or culture medium without serum and antibiotics. For very small volumes, we suggest to pre-dilute DreamFect Gold in deionized water.
   c. Combine the two solutions, mix gently by pipetting up and down and incubate the mixture for 20 minutes at room temperature. Do not vortex.

Table 3: Suggested dilution procedure and amount of siRNA to test:

Culture vessel	96-well		24-well		12-well		6-well	
Dilution serum-free medium or PBS	25µL		25µL		25µL		50µL	
Amount of siRNA (1 µM stock)*								
Final siRNA concentration	**µL**	**ng**	**µL**	**ng**	**µL**	**ng**	**µL**	**ng**
20 nM	4	54	10	135	20	270	40	540
50 nM	10	135	25	337.5	50	675	100	1350

Table 4: Recommended amount of DreamFect™ Gold per nM of siRNA used:

Culture vessel	96-well	24-well	12-well	6-well
Dilution serum-free medium or PBS	25µL	25µL	25µL	50µL
	Amount of DreamFect Gold			
Final siRNA concentration				
20 nM	0.3 µL	1 µL	2 µL	4 µL
50 nM	0.5 µL	2 µL	4 µL	8 µL

3) ***Transfection***. Add the mixture drop by drop directly onto the cells. The total transfection volume per well is indicated in table 1 (culture medium + complexes solution).

4) ***Assay***. Incubate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of gene silencing. Depending on the siRNA amount, the gene targeted and the cell type, assays can be monitored 24 to 96h post-transfection. We recommend 24h for RNA analysis and 48h to 72h for protein knockdown analyses.
   - 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:

A. 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.
B. 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.
C. Start with 50 nM siRNA concentration and test four amounts of DreamFect Gold™.
D. 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.
E. 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.

3.6. Co-Transfection siRNA-DNA Protocol

DreamFect™ Gold is also suitable for co-transfection of siRNA and plasmid DNA. Important considerations before beginning transfection:

- The correct choice of optimal plating density also 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.
- Always use a volume of reagent that is at least twice the quantity of nucleic acid (2µL per µg of DNA).
- For co-transfection of several plasmids DNA, mix the same amount of each plasmid and transfect as described above in section 3.3 or 3.4. For instance, if you have two DNA plasmids, mix 2.5 µg of each plasmid, complex the 5 µg of DNA with at least 10 µL of DreamFect Gold™ (20µL recommended).
- The DNA, siRNA and DreamFect™ Gold solutions should have an ambient temperature and be gently vortexed prior to use

1) Plate the cells as described in section 3.2, Table 1 for adherent cells and section 3.4, Table 2 for suspension cells.

2) Preparation of the complexes. Two options are possible for co-transfection.

   a. Option 1: Prepare the DreamFect Gold / DNA and DreamFect Gold / siRNA complexes separately in 2 different tubes.
      i. Prepare the DreamFect Gold / DNA complexes as described in section 3.3, incubate 15 minutes.

ii. Prepare the DreamFect Gold / siRNA complexes as described in section 3.5, incubate 15 minutes.
iii. Combine the solutions of DreamFect Gold / DNA with DreamFect Gold / siRNA, mix gently by pipetting up and down (do not vortex) and add directly to the cells.

Or

b. Option 2: Prepare the DreamFect Gold / nucleic acid complexes by pooling the DNA and siRNA together and then add the transfection reagent.
   i. Dilute the siRNA and DNA together in 1 tube (as described above).
   ii. Prepare the DreamFect Gold solution in a separate tube. Add sufficient amount of DreamFect for both DNA and siRNA. As a starting point, we suggest using a DreamFect Gold / total nucleic acid (DNA and siRNA) ratio of **5µL DreamFect Gold per µg of total nucleic acids**.
   iii. Mix gently the two solutions by carefully pipetting up and down and incubate the mixture for 15-20 minutes at room temperature. Do not vortex!

3) ***Transfection***. Add the complexes drop by drop onto the cells growing in serum-containing culture medium and homogenize by gently rocking the plate side to side to ensure a uniform distribution of the mixture.

4) ***Assay***. Incubate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of gene knockdown. Depending on the siRNA amount, the gene targeted and the cell type, assays can be monitored 24 to 96h post-transfection. We recommend 24h for RNA analysis and 48h to 72h for protein knockdown analyses.

- **For some cells, 24 hours post-transfection replace the old media with fresh media or just add fresh growth culture medium to the cells. * (see remark in section 3.3.5)**
- **In the case of cells very sensitive to transfection, the medium can be changed after 3-4 hours or 24 hours incubation with fresh medium. * (see remark in section 3.3.5)**

Options for Co-transfection
Prepare the cells and complexes as described above (steps 1 to 2). **Step 3** can be realized sequentially instead of simultaneously. So, cells can be transfected with siRNA first and 4 to 24h later be transfected with DNA. Follow the procedure as detailed above for DNA and siRNA transfection (3.3 or 3.4 and 3.5). A medium changed can be also performed before the DNA transfection.

3.7. Optimization Protocol

Although high transfection efficiencies can be achieved in a broad range of cell types with the rapid protocol, some optimization may be needed in order to obtain the maximum efficiency in particular cells. For best results, we recommend optimization of 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 (DNA/siRNA/RNA)
- 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 (siRNA 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 5. You can test ratios from 1 to 6 µl of DreamFect™ Gold reagent per 1 µg DNA.

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 6. The reagents can be pre-diluted in deionized water and resulting aliquots are incubated with siRNA. Diluted DreamFect™ Gold solution must be freshly prepared.

Table 5: Suggested range of DreamFect™ Gold for optimization.

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
12 well	1	1 – 6	1 - 2 – 3 – 4 - 5 – 6
6 well	2	2 – 12	2 - 4 – 6 - 8 – 12
60 mm dish	5	5 – 30	5 – 10 – 15 – 20 – 25 – 30
90 - 100 mm dish	10	10 – 60	10 – 20 – 30 – 40 – 50 – 60
T-75 flask	15	15 – 90	15 – 30 – 45 – 60 – 75 – 90

Table 6: Recommended amount of DreamFect™ Gold per nM of siRNA used:

Culture vessel	96-well	24-well	12-well	6-well
Final transfection volume	200 µL	500 µL	1 mL	2 mL
		Amount of Lullaby®		
Final siRNA Conc.				
25 nM	0.15 – 0.3 – 0.45 – 0.6µL	0.5 – 1 – 1.5 – 2µL	1 – 2 – 3 – 4µL	2 – 4 – 6 – 8µL
50 nM	0.25 – 0.5 – 0.75 – 1µL	1 – 2 – 3 – 4µL	2 – 4 –- 6 – 8µL	4 – 8 – 12 – 16µL

3) Quantity of DNA or siRNA:

To achieve the optimum transfection efficiency, the amount of DNA or the concentration of siRNA used can be increased. However a high amount of the complexes can result in over expression or lysis of the cells. These effects vary with the number of cells so, it is important to always keep the number of cells and the incubation time constant during your optimization procedure. Thus, after optimization of the DreamFect™ Gold / DNA or DreamFect™ Gold / siRNA ratios, proceed to adjust the best amount of DNA or the best concentration of siRNA required by maintaining a fixed ratio of DreamFect™ Gold reagent to DNA or siRNA, and vary the DNA quantity over the suggested range (table 7).

Table 7: Suggested range of DNA amounts for optimization.

Tissue Culture Dish	DNA Quantity (µg)	Transfection Volume
96 well	0.1 – 0.5	200 µL
24 well	0.5 – 2	500 µL
12 well	1 – 4	1 mL
6 well	2 – 10	2 mL
60 mm dish	5 – 30	4 mL
T-75 flask	15 – 90	10 mL

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 the DNA/RNA/siRNA and DreamFect™ Gold solutions 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, 27mM 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 ratio and DNA amount 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.

For stable transfection, cells can be seeded with lower density and, taking into account the efficiency of DreamFect™ Gold, the quantity of DNA used can be reduced. 48 to 72 hours post-transfection, cells are transferred to fresh medium containing the appropriate antibiotics for selection. It is important to wait at least 48 hours before exposing the transduced cells to selection media. For some cell types it may be necessary to wait as long as 4 to 5 days before applying the selection condition.

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 hours of incubation is done. Consequently, the cells may be kept in serum-free or reduced serum conditions during the first 4 hours 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 hours by analyzing the gene product. Reporter genes such as GFP, β-galactosidase, secreted alkaline phosphatase or luciferase can be used to quantitatively measured gene expression. For example, percentage of cells expressing the β-galactosidase transgene can be visualized by histochemical staining with X-Gal (catalog number # GX10003).

OZ Biosciences team has developed a detail protocol for optimization and also cell specific optimal transfection procedure. Thus, do not hesitate to contact our technical service at tech@ozbiosciences.com to request these specific protocols.

4. Appendix

4.1 Quality Controls

To assure the performance of each lot of DreamFect Gold™ produced, we qualify each component using rigorous standards. The following *in vitro* assays are conducted to qualify the function, quality and activity of each kit component.

Specification	Standard Quality Controls
Purity	Silica Gel TLC assays. Every compound shall have a single spot.
Sterility	Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 7 days.
Biological Activity	Transfection efficacies on NIH-3T3 and COS 7 cells. Every lot shall have an acceptance specification of > 80% of the activity of the reference lot.

4.2. Troubleshooting

Problems	Comments and Suggestions
Low transfection efficiency	1- **DreamFect Gold / nucleic acid ratio.** Optimize the reagent / nucleic acid ratio by using a fixed amount of DNA (µg) or siRNA (nM) and vary the amount of DreamFect Gold from 2 times less up to three times more than the suggested amount detailed in the Table 5 and 6. 2- **DNA amount.** Use different quantity of DNA with the recommended or optimized (above) transfection reagent / DNA ratio. 3- **Cell density.** A non-optimal cell density at the time of transfection can lead to insufficient uptake. The optimal confluency should range from 50 to 70% (true confluency, corresponding to 90% visual confluency) but most favorable cell density may vary according to the cell type; preferably mid-log growth phase. 4- **DNA or siRNA quality.** Nucleic acids should be as pure as possible. Free of contaminants (proteins, phenol, ethanol etc.) and endotoxins levels must be very low since they interfere with transfection efficiencies**.** Employ nuclease-free materials. 5- **Type of promoter**. Ensure that DNA promoter can be recognized by the cells to be transfected. Another cells or viral-driven reporter gene expression can be used as a control. 6- **Cell condition.** 1) Cells that have been in culture for a long time (> 8 weeks) may become resistant to transfection. Use freshly thawed cells that have been passaged at least once. 2) Cells should be healthy and assay during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) alters considerably the transfection efficiency.

Low transfection efficiency	7- **Medium used for preparing DNA / transfection reagent complexes**. It is critical that serum-free medium or buffer (HBS, PBS) are used during the preparation of the complexes. Avoid any direct contact of pure DreamFect Gold and pure nucleic acid solution with the plastic surface. 8- **Cell culture medium composition.** 1) For some cells, transfection efficiency can be increased without serum or under reduced serum condition. Thus, transfect these cells in serum-free medium during the first 4h of incubation. 2) The presence of antibiotics might affect cell health and transfection efficiency. 9- **Incubation time and transfection volume.** 1) The optimal time range between transfection and assay varies with cells, promoter, expression product, etc. The transfection efficiency can be monitored after 24 – 96h by analyzing the gene product. Several reporter genes can be used to quantitatively monitored gene expression kinetics. 2) To increase transfection efficiency, transfection volume suggested can be reduced for the first 24 hours. 10- **Old transfection reagent / DNA complexes.** The transfection reagent / DNA complexes must be freshly prepared every time. Complexes prepared and stored for longer than 1 hour can be aggregated. 11- **Transgene detection assay**. Ensure that your post-transfection assay is properly set up and includes a positive control. 12- **Transfection reagent temperature.** Reagents should have an ambient temperature and be vortexed prior to use. 13- **Transfection reagent storage.** Transfection efficiency can slowly decrease if DreamFect Gold is kept more than one week at +4°C. Store at -20°C to recover initial efficiency.
Cellular toxicity	1- **Unhealthy cells.** 1) Check cells for contamination, 2) Use new batch of cells, 3) Ensure culture medium condition (pH, type of medium used, contamination etc), 4) Cells are too confluent or cell density is too low, 5) Verify equipments and materials 2- **Transgene product is toxic.** Use suitable controls such as cells alone, transfection reagent alone or mock transfection with a DNA or siRNA control. 3- **siRNA/DNA quality - Presence of contaminants.** Ensure that nucleic acid is pure, contaminant-free and endotoxin-free. Use high quality nucleic acids as impurities can lead to cell death. 4- **Concentration of transfection reagent / nucleic acid too high.** Decrease the amount of nucleic acid / reagent complexes added to the cells by lowering the nucleic acid amount or the transfection reagent concentration. Complexes aggregation can cause some toxicity; prepare them freshly and adjust the ratio as outlined previously. 5- **Incubation time.** Reduce the incubation time of complexes with the cells by replacing the transfection medium by fresh medium after 4h to 24h. 6- **Key gene silencing.** If the targeted gene is essential for cell survival or if a key gene is non-specifically silenced by the siRNA, this can lead to cell death.
No or weak gene silencing effect	1- **siRNA design.** The design of an efficient siRNA is a crucial step. Ensure to use a validated siRNA sequence. If a validated siRNA cannot be used, assay your sequence in an easy to transfect cell line (if possible) in order to validate it (HeLa cells for example). 2- **siRNA concentration.** Use higher amount of siRNA. 3- **Incubation time.** Perform a time-course experiment to set up the optimal incubation time since gene silencing is dependent on the gene expression and the protein turnover rate. 4-**Medium used for preparing transfection reagent/siRNA complexes**. It is critical that serum-free medium or buffer (HBS, PBS) are used during the complexes preparation. 5- **Old DreamFect Gold/siRNA complexes**. The DreamFect Gold / siRNA complexes must be freshly prepared every time. Complexes kept for longer than 1 hour can be aggregated.

Our dedicated and specialized technical support group will be pleased to answer any of your requests and to help you with your transfection experiments. tech@ozbiosciences.com. In addition, do not hesitate to visit our website www.ozbiosciences.com and the FAQ section.

Description	Reference
Tee-Technology (lipid-based reagents)	
Lullaby siRNA transfection reagent	LL71000
DreamFect Transfection reagent 1mL	DF41000
EcoTransfect Transfection Reagent 1mL	ET11000
VeroFect Transfection Reagent 1mL	VF61000
FlyFectin Transfection Reagent 1mL	FF51000
Magnetofection Technology	
Super Magnetic Plate	MF10000
Magnetic Plate 96-magnets	MF10096
PolyMag 1mL	PN31000
CombiMag 1mL	CM21000
SilenceMag 1mL	SM11000
ViroMag 1mL	VM41000
ViroMag R/L 1mL	RL41000
FluoMag-P 100µL	FP10100
FluoMag-C 100µL	FC10100
FluoMag-S 100µL	FS10100
FluoMag-V 100µL	FV10100
Gene & Protein Tools	
Bradford – Protein Assay Kit	BA00100
GeneBlaster™ Ruby	GB20011
GeneBlaster™ Sapphire	GB20012
GeneBlaster™ Topaz	GB20013
β-Galactosidase (ONPG) assay kits	GO10001
β-Galactosidase (CPRG) assay kits	GC10002
X-Gal Staining Kit	GX10003
DNA markers	
100 bp DNA ladder	PF00100
100 bp DNA ladder PLUS	PF00200
1 Kbp DNA ladder	PF00300
ShortRun DNA Marker	PF00400
pBR328 Hinf I / Bgl I	PF00500
pUC18 Hpa II	PF00600
pUC19 MSp I	PF00700
pBR322 Hae III	PF00800
Λ Hind III / phiX 174 Hae III	PF00900

Please, feel free to contact us for all complementary information and remember to visit our website to stay informed on the latest breakthrough technologies and updated on our complete product list.

Limited License

The purchase of the DreamFect™ Gold Reagent grants the purchaser a non-transferable, non-exclusive license to use the kit and/or its separate and included components (as listed in section 1, Kit Contents). This reagent is intended **for in-house research only** by the buyer. Such use is limited to the transfection of nucleic acids as described in the product manual. In addition, research only use means that this kit and all of its contents are excluded, without limitation, from resale, repackaging, or use for the making or selling of any commercial product or service without the written approval of OZ Biosciences.

Separate licenses are available from OZ Biosciences for the express purpose of non-research use or applications of the DreamFect™ Gold Reagent. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact the Director of Business Development at OZ Biosciences.

Buyers may end this License at any time by returning all DreamFect™ Gold Reagent material and documentation to OZ Biosciences, or by destroying all DreamFect™ Gold components. Purchasers are advised to contact OZ Biosciences with the notification that a DreamFect™ Gold kit is being returned in order to be reimbursed and/or to definitely terminate a license for internal research use only granted through the purchase of the kit(s).

This document covers entirely the terms of the DreamFect™ Gold Reagent research only license, and does not grant any other express or implied license. The laws of the French Government shall govern the interpretation and enforcement of the terms of this License.

Product Use Limitations

The DreamFect™ Gold Reagent and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.

For more information, or for any comments on the terms and conditions of this License, please contact:

Director of Business Development
OZ Biosciences
Parc Scientifique et Technologique de Luminy
BP13
13273 Marseille Cedex 9, France
Tel: +33 (0) 4.91.82.81.74
Fax: +33 (0) 4.91.82.81.70
E-mail: contact@ozbiosciences.com