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BOCA SCIENTIFIC

Transfection reagent

LipoMag Kit

Improve Transfection Efficiency

Protocol

Magnetofection Technology
This reagent needs to be used with a magnetic plate

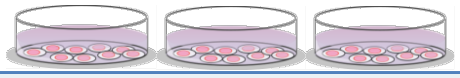
OZBIOSCIENCES
The art of delivery systems

LipoMag Kit Quick Protocol

To find the ideal conditions, DreamFect™ Gold must be tested at ratios **2 µL/µg**, **3 µL/µg** and **4 µL/µg**. µL of DreamFect Gold / µg of DNA); CombiMag must be used at **1µL /µg** of DNA. For the DNA quantity, we suggest **0.125 µg** per well in 96-well, **0.5 µg** per well in 24-well and **2 µg** per well in 6-well.

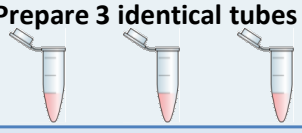
1

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
0.125µg in 25µL of serum-free medium or buffer* x 3	0.5µg in 50µL of serum-free medium or buffer* x 3	2µg 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
0.25µL/0.375µL/0.5µL in 25µL of serum-free medium or buffer*	1µL/1.5µL/2µL in 50µL of serum-free medium or buffer*	4µL/6µL/8µL in 100µL of serum-free medium or buffer*

4

Prepare 3 identical tubes of CombiMag™

96 well plate	24 well plate	6 well plate
0.125µL in an empty microtube x 3	0.5µL in an empty microtube x 3	2µL in an empty microtube x 3

5

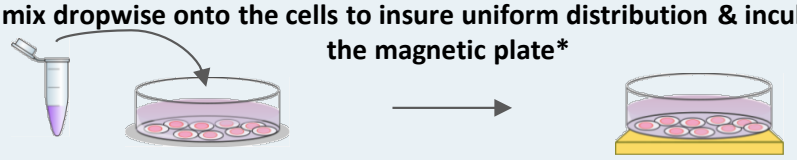
Mix each DNA tube (step 2) to each DreamFect™ Gold tube (step 3) & proceed to next step

6

Add each mix to a tube of CombiMag (step 4) and incubate 20 min at room temperature

7

Distribute each mix dropwise onto the cells to insure uniform distribution & incubate the cells 20 min on the magnetic plate*

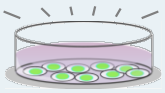


8

Remove the cells from the magnetic plate and incubate cells for 24 to 72h at 37°C until evaluation of transgene expression*

9

Choose the best ratio DNA : DreamFect™ Gold : CombiMag



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/CombiMag 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 reagents with deionized water for doses less than 1µL.

- ✓ For most cell types, a medium change is not required after Magnetofection. However, it may be necessary for cells that are sensitive to serum/supplement concentration. This can be done immediately after the 20min. incubation on the magnetic plate while keeping the cells onto the magnetic device, or 4 to 6h post-Magnetofection. Alternatively, the cells may be kept in serum-free medium during Magnetofection (up to 4h). In this case, a medium change will be required after Magnetofection.

- ✓ Do not freeze the magnetic nanoparticles CombiMag.

LipoMag Kit Reagent | Specifications

Package content	LM80250: 500 µL of DreamFect Gold + 250 µL of CombiMag LM80500: 1 mL of DreamFect Gold + 500 µL of CombiMag LM80000: 500 µL of DreamFect Gold + 250 µL of CombiMag + Super Magnetic Plate
Shipping conditions	Room Temperature
Storage conditions	Upon reception, store the DreamFect Gold reagent at -20°C and the CombiMag at +4°C
Shelf life	1 year from the date of purchase when properly stored and handled
Product Descriptions	LipoMag Kit is a magnetic nanoparticles formulation specifically designed to achieve high transfection efficiency of primary cells, hard-to-transfect cells and cell lines.
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 (refer to 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	DNA Quantity (µg)	DreamFect Gold Volume (µL)	Dilution Volume (µL)	CombiMag Volume (µL)	Transfection Volume
96 well	0.05 – 0.2 x 10^5	0.125	0.375	2 x 25	0.125	200 µL
24 well	0.5 – 1 x 10^5	0.5	1.5	2 x 50	0.5	500 µL
6 well	2 – 5 x 10^5	2	6	2 x 100	2	2 mL

Table 1: Suggested cell number, DNA amount, DreamFect Gold,CombiMag volumes and transfection conditions

2. DNA/LipoMag Kit complexes preparation

a. *DNA*: Dilute the indicated quantity of DNA (refer to Table 1) in 25 to 100 µL of culture medium without serum and supplement.

b. *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 1).

c. *CombiMag*: Vortex the CombiMag and place the appropriate amounts in an empty microtube (refer to Table 1).

d. Add DNA solution to DreamFect Gold solution, mix gently by carefully pipetting up and down and incubate the mixture at room temperature for 5 minutes. Do not vortex

e. Add the mix to CombiMag reagent and gently mix by pipetting up & down and incubate 20 minutes at room temperature.

3. Transfection

a. Add the complexes (DNA/DreamFect Gold/CombiMag) onto cells drop by drop and gently rock the plate to ensure a uniform distribution.

b. Place the cell culture plate on the magnetic plate during 30 minutes.

c. Remove the magnetic plate.

d. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of transgene expression (from 24h up to 72h).

NOTE: in case of cells very sensitive to transfection, the medium can be changed after 3-4 hours or 24 hours 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 (refer to Table 2).

Tissue Culture Dish	Suspension Cell Number	DNA Quantity (µg)	DreamFect Gold Volume (µL)	CombiMag Volume (µL)	Dilution Volume (µL)	Transfection Volume
96 well	0.5 – 1 x 10^5	0.5	1.5	0.5	2 x 25	100 µL
24 well	2 - 4 x 10^5	2	6	2	2 x 50	250 µL
6 well	10 - 15 x 10^5	6	18	6	2 x 100	1 mL

Table 2: Suggested transfection conditions for suspension cells

2. DNA/LipoMag Kit complexes preparation

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

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

c. *CombiMag*: Vortex the CombiMag and place the appropriate amounts in an empty microtube (see Table 2).

d. Add the DNA solution to the DreamFect Gold solution, mix gently by carefully pipetting up and down and incubate at room temperature for 5 minutes.

e. Combine the DNA/MTX solution with the CombiMag reagent, mix gently by pipetting up & down and incubate 20 minutes at room temperature.

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 2. 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. Perform one of the following three options to sediment the cells at the bottom of the culture dish in order to promote the contact with the magnetic nanoparticles.

1. Seed the cells on polylysine-coated plates and use the protocol for adherent cells

OR

2. Briefly, centrifuge the cells (2 minutes) to pellet them and use the protocol for adherent cell

OR

3. Mix cell suspension with 30 µL of *CombiMag* reagent per mL of cell suspension.
   - Incubate for 10 - 15 minutes.
   - Distribute cells to your tissue culture dish placed upon the magnetic plate (volume of culture medium containing cells depends on the culture dish size; see suggested transfection volume in table above as indication).
   - Incubate for 15 minutes

OR

4. Incubate the cells in serum free medium during 2 hours prior Magnetofection. The absence of serum allows some cells to adhere onto the plastic dish surface.

c. Next, place the cells onto the magnetic plate and add the complexes directly onto the cells dropwise.

d. Incubate for 30 minutes and remove the magnetic plate.

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

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

g. Incubate the cells at 37°C in a CO2 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.

NOTE: depending on the cell type, 24 hours post-transfection replace the old media with fresh media or just add fresh growth culture medium to the cells.

IMPORTANT OBSERVATIONS

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

- We suggest 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.
- GeneBlaster™ Topaz (catalog # GB20013) can be used to boost the gene expression level in some cell lines.

Protocol | 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 0.5 µg of each plasmid, complex the 1 µg of DNA with 3 µL of DreamFect Gold and 1 µL CombiMag, composing the LipoMag Kit.

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 protocols 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.

1. Cell 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 / LipoMag Kit complexes preparation

The siRNA, DreamFect Gold and CombiMag solutions should have an ambient temperature, be gently vortexed prior to use and be combined within 5 minutes.

a. *siRNA solution*. Dilute the siRNA stock solution (for instance 1µM) in 50 or 100 µL (see Table 3) of PBS or culture medium without serum. We advise starting with a final siRNA concentration of 50nM.

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 3: Suggested dilution procedure and amount of siRNA to test

b. *DreamFect Gold preparation*. Dilute the DreamFect Gold in 50 or 100 µL (see Table 4) of PBS or culture medium without serum.

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
Final siRNA concentration	*Amount of DreamFect Gold (µL)*		
10nM	0.15	0.5	2
20nM	0.3	1	4
≥ 50 nM	0.5	2	8

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

c. Combine the two solutions, mix gently by pipetting up and down and incubate the mixture for 5-10 minutes at room temperature.
Do not vortex.

d. Prepare a tube containing 1 µL of CombiMag per µg siRNA.

e. Add DreamFect Gold / siRNA complexes to the CombiMag solution and incubate 20 min at room temperature in an empty microtube.

3. Transfection

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

b. Place the cell culture plate upon the magnetic plate during 30 minutes

c. Remove the magnetic plate.

d. Cultivate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of gene silencing. 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 50nM siRNA and test four amounts of DreamFect Gold with a fixed ratio of CombiMag.

- 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 /DreamFect Gold / CombiMag. On day two, change your medium and repeat the treatment with 25nm siRNA / DreamFect Gold / CombiMag.

Protocol Optimization

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

- Ratio of DreamFect™ Gold to nucleic acid
- Ratio of CombiMag 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 (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.

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 5: 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 6. Diluted DreamFect™ Gold solution in deionized water for volumes less than 1 µL must be freshly prepared.

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
Final siRNA concentration	*Amount of DreamFect Gold (µL)*		
25nM	0.15 – 0.3 – 0.45 – 0.6	0.5 – 1 – 1.5 - 2	2 – 4 – 6 - 8
≥ 50 nM	0.25 – 0.5 – 0.75 - 1	1- 2 – 3 - 4	4 – 8 – 12 - 16

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

3. CombiMag / nucleic acid ratio

Once the DreamFect™ Gold to nucleic ratio is found, CombiMag volume may be adjusted in order to reach the maximum efficiency. Use 0.5 / 1 or 2 µL of CombiMag per µg of nucleic acid.

4. 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. (refer to Table 7).

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

Table 7: Suggested range of DNA amounts for optimization

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

5. 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.

6. 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.

For stable transfection, cells can be seeded with lower density and, taking into account the efficiency of LipoMag Kit, 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.

7. Effect of serum /Transfection volume

Almost all cell lines transfected with LipoMag Kit 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.

8. 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).

Additional products for primary and hard-to-transfect cells experiments

- **SilenceMag** for siRNA applications
- **NeuroMag** for transfections of neurons
- ***In vivo* PolyMag** for *in vivo* applications

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