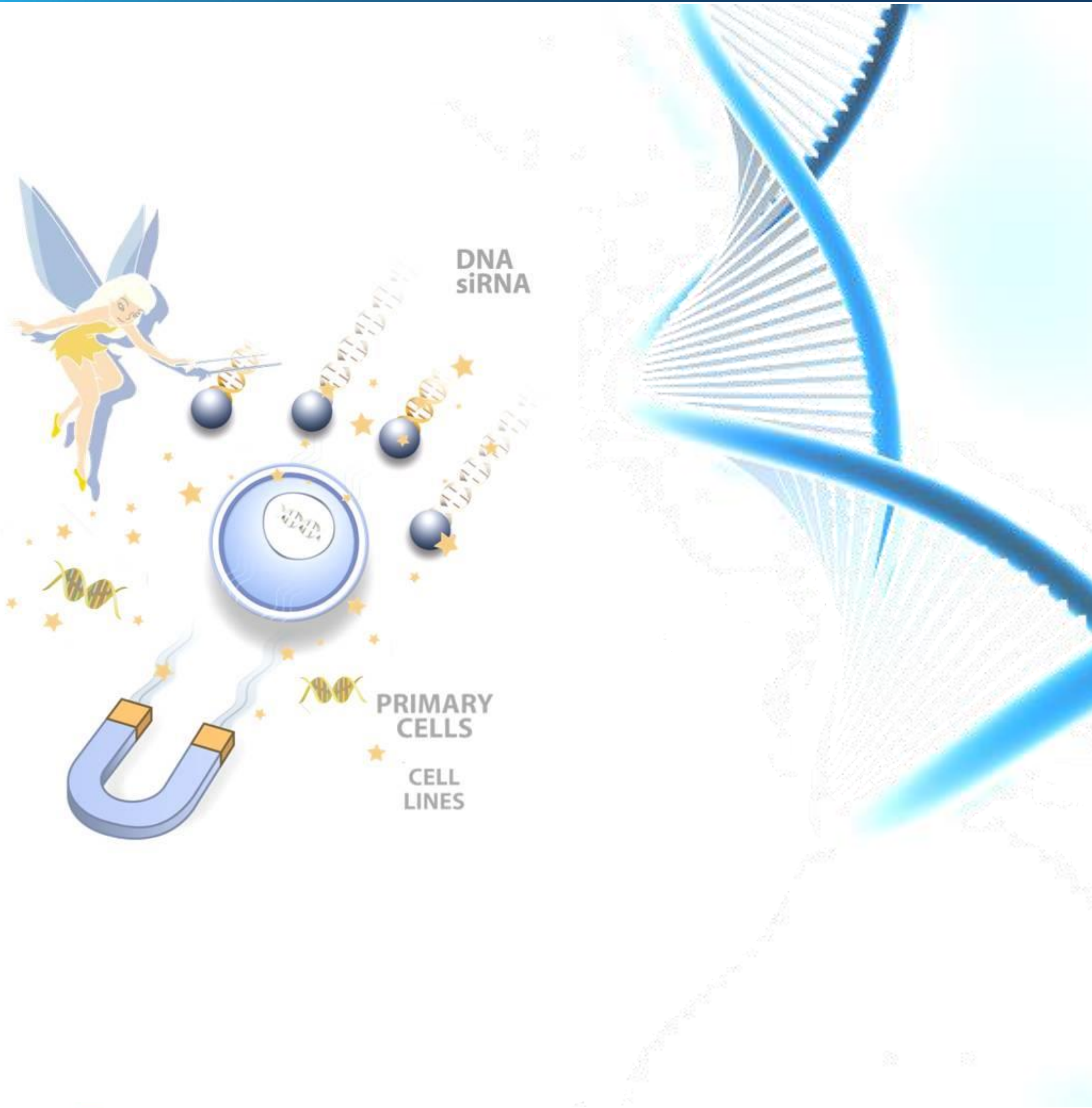
Magnetofection™ LipoMag Kit

INSTRUCTION MANUAL



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The art of delivery systems

LipoMag Kit

Instruction Manual

LipoMag Kit

The newest generation of outstanding transfection reagent that achieves higher transfection efficiency and superior transgene expression level now associated Magnetofection™ (CombiMag) and Lipofection (DreamFect™ Gold).

List of LipoMag Kits

Catalog Number	Description	Volume (µL)	Size (number of transfection / µg of DNA)
LM80250	250µL of CombiMag + 500µL of DreamFect Gold	250µL +500µL	250
LM80500	500µL of CombiMag + 1mL of DreamFect Gold	500µL + 1mL	500
LM80000	250µL of CombiMag + 500µL of DreamFect Gold + 1 super magnetic plate	250µL + 500µL	250

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: order@ozbiosciences.com) or directly through our website. For all other supplementary information, do not hesitate to contact our dedicated technical support: tech@ozbiosciences.com.

OZ Biosciences SAS
163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09 - FRANCE
Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515
contact@ozbiosciences.com
order@ozbiosciences.com

OZ Biosciences INC
4901 Morena Blvd,
Suite 501
San Diego CA 92117 - USA
Ph : + 1-858-246-7840
Fax : + 1-855-631-0626
contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com

www.ozbiosciences.com

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1. Technology

1.1. Description

Congratulations on your purchase of the ***LipoMag Kit*** transfection reagent!

LipoMag Kit is the latest transfection tool that associates the powerful Magnetofection reagent, CombiMag, with the efficient Lipofection reagent, DreamFect Gold. ***DreamFect™ Gold*** is based on the **Tee-Technology ("Triggered Endosomal Escape")** which combines and exploits the properties of cationic lipids and polymers to achieve an efficient DNA delivery into cells. ***Magnetofection™*** is an original, simple and highly efficient method to transfect cells in culture and *in vivo*. The magnetic force exerted upon gene vectors associated with magnetic nanoparticles to drive vector towards the target cells allows the vector dose to get concentrated onto the cell within minutes. Because the level of transgene expression matters as well as the transfection efficiency, OZ Biosciences has now combine the new generation of lipopolyamines with improved cytoplasmic release process and better biodegradability, ***DreamFect™ Gold***, with the universal ***CombiMag***. In this way, high transfection efficiency combines with superior transgene expression level are achieved. LipoMag Kit can be used with all types of nucleic acids and allows their delivery in a wide variety of cell lines and primary cells.

Principal **LipoMag Kit** advantages:

1. Highest efficiency
2. Achieves greater transgene expression level than any other reagents
3. Enhances DreamFect Gold Efficiency and outperforms competitors
4. Complete Biodegradability
5. Universal (primary cells and cell lines)
6. Multipurpose (various types of nucleic acid)
7. Simple, ready-to-use & rapid
8. Serum compatible

1.2. Kit Contents

OZ Biosciences offers two sizes of LipoMag Kit:

- One Kit containing 500 µL of DreamFect™ Gold and 250 µL of CombiMag suitable for 250 transfections with 1 µg of DNA
- One Kit containing 1 mL of DreamFect™ Gold and 500 µL of CombiMag suitable for 500 transfections with 1 µg of DNA

Stability and Storage

Storage: Upon reception and for long-term use, store DreamFect Gold reagent at -20°C and CombiMag at 4°C. LipoMag Kit are very stable for at least one year under recommended storage conditions. The DreamFect Gold 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

LipoMag Kit associates DreamFect™ Gold that have been developed for very efficient transfections of various types of nucleic acids such as **DNA, mRNA, siRNA or oligonucleotides** in a wide variety of cells and CombiMag, the magnetic nanoparticles designed to be combined with lipid reagents. These transfection reagents are serum compatible and can be used for transient and stable transfection. These products are very stable, ready-to-use and intended for research purpose only.

2.2. Cell Types

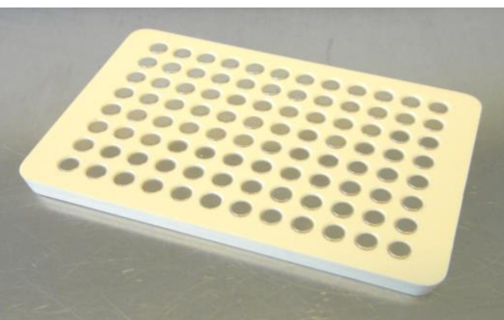
LipoMag Kit is suitable for numerous cell types. It has been successfully tested on a variety of immortalized cell lines as well as primary cells. An updated list of transfected cells is available on OZ Biosciences website: www.ozbiosciences.com. You can also submit your data to tech@ozbiosciences.com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

2.3. DreamFect™ Gold and Magnetofection™

Responding to our large customer demands, we are proud to announce you that DreamFect™ Gold reagent is now combined with our Magnetofection™ transfection technology (CombiMag) in one Kit. This product will be particularly useful for very difficult-to-transfect cells & primary cells. The combination of the two technologies allows you using very small amounts of DNA and increase the overall efficiency of your transfections. For further information concerning the Magnetofection™ technology, see our website: www.ozbiosciences.com.

3. Magnetofection™ Apparatus

Apart from suitable magnetic nanoparticles, Magnetofection™ requires appropriate magnetic fields. A magnetic plate especially designed for Magnetofection is provided to exert these specific magnetic fields. Its special geometry not only produces strong magnetic fields it is applicable for any plate formats (T-75 flasks, 60 & 100 mm dishes, 6-, 12- and 24-well plates). Super Magnetic Plate suits for all cell culture supports and Mega Magnetic Plate is designed to hold up to 4 culture dishes at one time. The magnetic plate design allows producing a heterogeneous magnetic field that magnetizes the nanoparticles in solution, forms a very strong gradient and covers all the surface of the plate.



Magnetic plate 96 magnets

Super Magnetic Plate

Mega Magnetic Plate

4. General Protocols

4.1. General Considerations

The instructions given below represent sample protocols that were applied successfully with a variety of cells. 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 LipoMag Kit Reagents) 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 **4.7)** 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 using **2 or 3 µL of DreamFect™ Gold / 1 µg of DNA with 1µL CombiMag.** You can use your routine culture medium for the transfection, except during preparation of the DreamFect™ Gold / DNA / CombiMag complexes (see **4.3** below).

- **Cells** should be healthy and assayed during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) or the cell activation state (endotoxins) will considerably affect the transfection efficiency. The cell proliferating rate is also critical 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 LipoMag Kit reagents 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 LipoMag Kit reagents 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 LipoMag Kit reagents and inversely. Each transfection reagent has its own molecular structure, biophysical properties and concentration, which have an important influence on their biological activity.

4.2. Cells Preparation

Adherent cells. It is recommended to seed or plate the cells the day prior transfection. Cells can also be prepared the same day of transfection (several hours before is suitable) the suitable cell density will depend on the growth rate and the conditions of the cells. Cells should be 60 %-90 % 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.

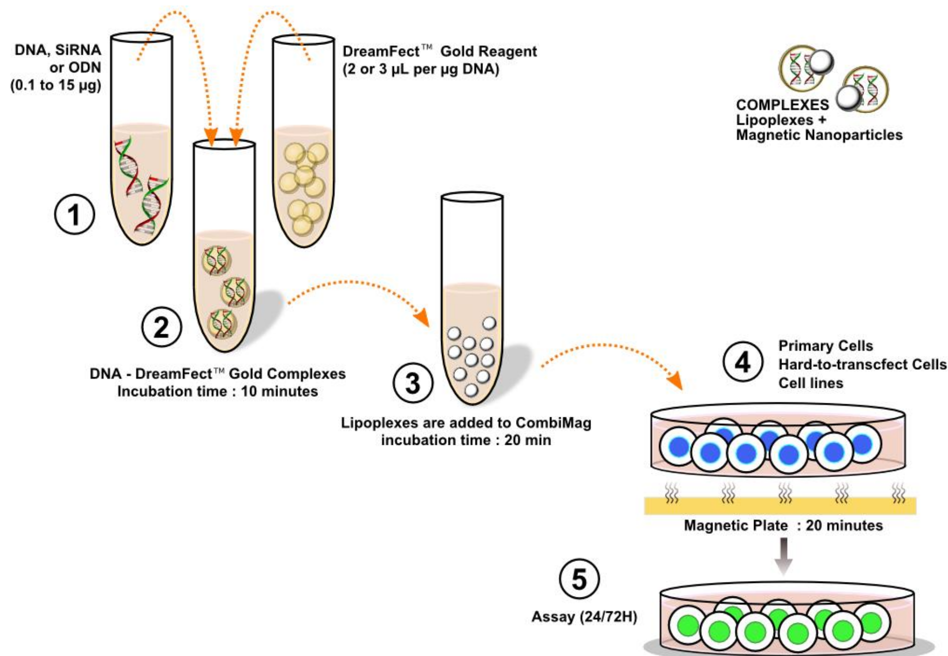
Suspension cells. For fast growing cells, split the cells the day before transfection at a density of 2 to 5 x 10^5 cells / ml, so they are in excellent condition on the day of transfection. (See section 4.4 for procedure)

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)	CombiMag Volume (µL)	Transfection Volume
96 well	0.05 – 0.2 x 10^5	0.25	0.5-0.75	2 x 25	0.25	200 µL
24 well	0.5 – 1 x 10^5	1	2-3	2 x 50	1	500 µL
12 well	1 – 2 x 10^5	2	4-6	2 x 50	2	1 mL
6 well	2 – 5 x 10^5	3	6-9	2 x 100	3	2 mL
60 mm dish	5 – 10 x 10^5	6	12-18	2 x 150	6	4 mL
90 - 100 mm	10 – 30 x 10^5	12	24-36	2 x 250	12	8 mL
T-75 flask	20 – 50 x 10^5	20	40-60	2 x 350	20	10 mL

4.3. Rapid Protocol – Adherent Cells



The DNA, DreamFect™ Gold and CombiMag solutions should have an ambient temperature and be gently vortexed prior to use. The rapid protocol is as simple as follows: Use 2-3 µL of DreamFect™ Gold per µg of DNA and 1µL of CombiMag. We suggest beginning with this ratio and optimize it, if required, by following section 4.7.

Important considerations before beginning transfection:

- CombiMag must be stored at +4°C and DreamFect™ Gold at -20°C. The storage at -20°C minimizes the size of liposomes and thus leads to higher efficiency. If transfections are performed routinely, DreamFect™ Gold can be kept at 4°C for one week after thawing; thereafter, it should be stored back at -20°C.
- Do not use serum-containing media for preparing the complexes!
- Prevent the DreamFect™ Gold, CombiMag 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) can result in materials lost by adsorption.

1) **a- DNA solution**. Dilute 0.25 to 20 µg of DNA in 25 to 350 µL of PBS* or culture medium without serum (see Table 1). (* for more information see section 4.7)

   b- 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.

   c- CombiMag solution. Before each use, vortex. Add 1 µL of CombiMag per µg of DNA to be transfected to a microtube. For DNA doses of less than 1 µg predilute an aliquot of CombiMag reagent with deionized water and use the volume required for your DNA dose. Discard the diluted CombiMag after use.

2) **Preparation of complexes**. 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 5-10 minutes at room temperature. Do not vortex or centrifuge!
   - The diluted solutions should be combined within 5 minutes.

3) Add the complexes (Lipoplexes) to the CombiMag Reagent, mix gently by carefully pipetting up and down 2-3 times and incubate the mixture for 20 minutes at room temperature.

4) **Transfection**. 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. Incubate the cells 20 minutes on the magnetic plate at 37°C in a CO_2 incubator.
   - At this step, a medium change may optionally be performed. Keep the magnetic plate beneath the cell culture dish, replace the medium with fresh medium and then remove the magnetic plate.

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 mixed with CombiMag are prepared in medium without serum or PBS because serum interferes with vector assembly. Then, the complexes cocktail is added to the cells that are covered with complete medium which 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.

4.4. Suspension Cells Protocol

The DNA, DreamFect™ Gold and CombiMag 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 4.7. The rapid protocol is as simple as follows: Use 3 µL of DreamFect™ Gold and 1µL of CombiMag 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) / CombiMag complexes as described above (section 4.3)
   - We suggest testing three DNA amounts while keeping the DNA/DreamFect Gold ratio constant (1/3).
3) 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

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.

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

OR

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

OR

c. Mix cell suspension with 30 µL of *CombiMag* reagent per mL of cell suspension.
   i. Incubate for 10 - 15 minutes.
   ii. 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).
   iii. Incubate for 15 minutes

OR

d. 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.

4) Next, add the magnetic complexes directly onto the cells dropwise and all over the well while keeping the cell culture plate on the magnetic plate. ***Important***: mix complexes with the cells by gently swirling the

plate (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.

5) Incubate for 15 minutes, remove magnetic plate.
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.

4.5. siRNA Transfection Protocol

1) The day prior transfection, prepare the cells as described in section 4.2. 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. 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. 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 5-10 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) Before each use, vortex CombiMag tube and add 1 µL of CombiMag per 1 µg siRNA in an empty microtube.
4) Add DreamFect™ Gold / siRNA complexes to the CombiMag solution and incubate 20 min at room temperature.
5) ***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).
6) Place the cell culture plate upon the magnetic plate for 15-20 minutes and remove the magnetic plate.
7) ***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 50nM siRNA and test four amounts of DreamFect Gold™ with a fixed ratio of CombiMag.
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 /DreamFect™ Gold / CombiMag. On day two, change your medium and repeat the treatment with 25nm siRNA / DreamFect™ Gold / CombiMag.

4.6. Co-Transfection siRNA/DNA or DNA/DNA Protocol

LipoMag Kit 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 4.3 or 4.4. 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) and add 5 µL of CombiMag.
- The DNA, siRNA, DreamFect™ Gold and CombiMag solutions should have an ambient temperature and be gently vortexed prior to use

1) Plate the cells as described in section 4.2, Table 1 for adherent cells and section 4.4, Table 2 for suspension cells.
2) Preparation of the Lipoplexes. 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 4.3, incubate 10 minutes.
      ii. Prepare the DreamFect Gold / siRNA complexes as described in section 4.5, incubate 10 minutes.
      iii. Combine the solutions of DreamFect Gold / DNA with DreamFect Gold / siRNA, mix gently by pipetting up and down (do not vortex).

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 **4µL 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 10 minutes at room temperature. Do not vortex!

3) Before each use, vortex CombiMag tube and add 1 µL of CombiMag per 1 µg nucleic acids in an empty microtube
4) Add DreamFect™ Gold / nucleic acids complexes to the CombiMag solution and incubate 20 min at room temperature.
5) ***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.
6) Place the cell culture plate upon the magnetic plate for 15-20 minutes and remove the magnetic plate.
7) ***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.
   - 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.

Options for Co-transfection

Prepare the cells and complexes as described above (steps 1 to 2). **Step 4** 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 (4.3 or 4.4 and 4.5). A medium changed can be also performed before the DNA transfection.

4.7. Optimization Protocol

Although high transfection efficiencies can be achieved in a broad range of cell types with LipoMag Kit 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 LipoMag Kit. Several parameters can be optimized:

- Ratio of DreamFect™ Gold to nucleic acid (DNA/siRNA/RNA)
- Ratio of CombiMag 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).
Once DreamFect™ gold ratio to nucleic acid is optimized, CombiMag ratio to nucleic acid may also be optimized.

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 50 nM	0.15 – 0.3 – 0.45 – 0.6µL 0.25 – 0.5 – 0.75 – 1µL	0.5 – 1 – 1.5 – 2µL 1 – 2 – 3 – 4µL	1 – 2 – 3 – 4µL 2 – 4 –- 6 – 8µL	2 – 4 – 6 – 8µL 4 – 8 – 12 – 16µL

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 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.

5) 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, 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 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 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).

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.

5. Appendix

5.1 Quality Controls

To assure the performance of each lot of LipoMag Kit 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.

5.2. Troubleshooting

Problems	Comments and Suggestions
Low transfection efficiency	1- **DreamFect Gold / nucleic acid ratio.** Optimize the 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 & 6. 2- **CombiMag / nucleic acid ratio**. Optimize the lipoplexes / CombiMag ratio by using the fixed optimized quantities of DreamFect Gold / DNA and varying the volume of CombiMag from 0.5 µL to 2 µL per µg of DNA. 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. 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.

6. Related Products

Our dedicated and specialized technical support group will be pleased to answer any of your request and to assist you in your experiments. Do not hesitate to contact us for all complementary information and remember to visit our website in order to stay inform on our last breakthrough technologies and updated on our complete product list. http://www.ozbiosciences.com.

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate *(standard size for all cell culture support)* Mega Magnetic plate *(mega size to hold 4 culture dishes at one time)*
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
Magnetofectamine™ kit: Lipofectamine™ 2000 + CombiMag (*for all nucleic acids*)
NeuroMag *(dedicated for neurons)*
SilenceMag *(for siRNA application)*
Transfection enhancer:
CombiMag *(to improve any transfection reagent efficiency)*
Viral Transduction enhancers:
ViroMag (*to optimize viral transduction*)
ViroMag R/L *(specific for Retrovirus and Lentivirus)*
AdenoMag *(for Adenoviruses)*
In vivo Magnetofection
In vivo ViroMag (for magnetic assisted viral infection)
In vivo PolyMag (polymer-based magnetic nanoparticles)
In vivo DogtorMag (lipid-based magnetic nanoparticles)
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
VeroFect *(for Vero cells)*
Ecotransfect *(Economical reagent for routine transfection)*
FlyFectin *(for Insect cells)*
i-MICST TECHNOLOGY
Viro-MICST (*to transduce directly on magnetic cell purification columns)*
3D TRANSFECTION TECHNOLOGY
3DfectIN *(for hydrogels culture)*
3Dfect *(for scaffolds culture)*
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit (*for **High Yield Protein Expression**)*
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN *(delivery reagent for antibodies)* Pro-DeliverIN *(delivery reagent for protein in vivo and in vitro)*
PLASMIDS PVECTOZ
pVectOZ-LacZ / pVectOZ-SEAP / pVectOZ-GFP / pVectOZ-Luciferase
ASSAY KITS
Bradford – Protein Assay Kit MTT cell proliferation kit β-Galactosidase assay kits (CPRG/ONPG)
BIOCHEMICALS
D-Luciferin, K^+ and Na^+ 1g G-418, Sulfate 1g
X-Gal powder 1g

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Director of Business Development
OZ Biosciences SAS
Parc Scientifique de Luminy
Zone Luminy Entreprise
163, avenue de Luminy – Case 922
13288 Marseille Cedex 9 - FRANCE
Ph: +33 (0)4. .86.94.85.16
Fax: +33 (0)4. .86.94.85.15
E-mail: business@ozbiosciences.com

CONTACTS

OZ Biosciences SAS
163 avenue de Luminy
Case 922, zone entreprise
13288 Marseille cedex 09
FRANCE

Ph: +33 (0) 486 948 516
Fax: +33 (0) 486 948 515

contact@ozbiosciences.com
order@ozbiosciences.com
tech@ozbiosciences.com

OZ Biosciences INC
4901 Morena Blvd,
Suite 501
San Diego CA 92117
USA

Ph : + 1-858-246-7840
Fax : + 1-855-631-0626

contactUSA@ozbiosciences.com
orderUSA@ozbiosciences.com
techUSA@ozbiosciences.com

www.ozbiosciences.com

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