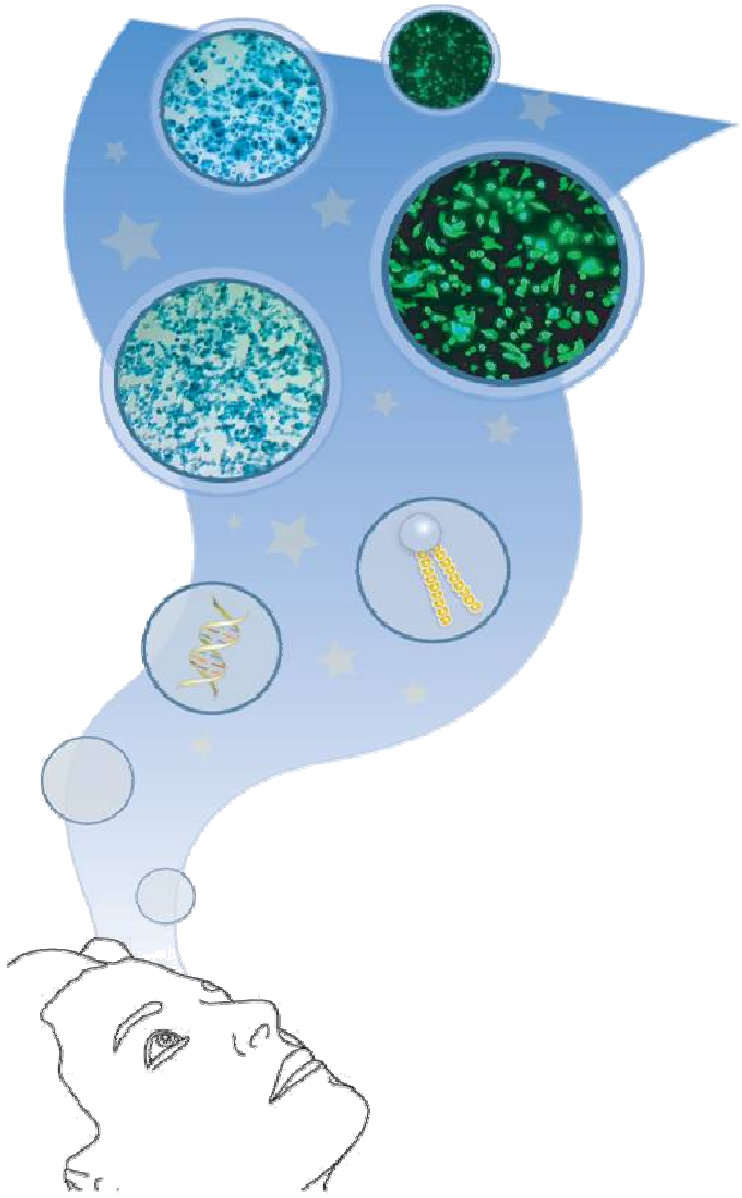
DreamFect™

INSTRUCTION MANUAL



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DreamFect ™

Instruction Manual

DreamFect™
The next generation of outstanding transfection reagent

List of DreamFect™ Kits

Catalog Number	Description	Volume (µL)	Size (number of transfection / µg of DNA)
DF40500	DreamFect ™	500	125
DF41000	DreamFect ™	1000	250
DF45000	DreamFect ™	5 X 1000	1250

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.

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1. Technology

1.1. Description

Congratulations on your purchase of the DreamFect™ transfection reagent!
DreamFect™ is a powerful transfection reagent based on the **Tee-Technology ("Triggered Endosomal Escape")**. The cationic lipids (lipoplexes) and polymers (polyplexes) are the most employed non-viral gene delivery systems. The Tee-Technology combines and exploits the properties of both entities to achieve an extremely efficient DNA delivery into cells. Indeed, DreamFect™ is the new generation of lipopolyamines; it contains a lipophilic part, like lipids, and a charged polyamine moiety, like cationic polymers. The association by electrostatic interactions of nucleic acids with the DreamFect™ reagent results in the tight compaction and protection of the-DNA. Then, these positively charged complexes bind to cell surface and are taken up by endocytosis. Inside the endosomes, the hydrophobic property of lipids acts in synergy with endosomes buffering capacity of the polycationic residues allowing: 1) a very efficient destabilization of the endosomal membrane 2) the release of large DNA amounts in the cytosol and 3) the DNA nuclear uptake. DreamFect™ can be used with plasmid-DNA, mRNA, siRNA or oligonucleotides and allows their delivery in a wide variety of cell lines or primary cells.

Principal DreamFect™ advantages:

1. Compaction of DNA in nanoparticles efficiently internalized by cells
2. Protection of nucleic acids against nucleases degradation
3. Efficient membrane destabilization and DNA delivery into cells
4. Highly efficient with low amounts of nucleic acids – Ideal for High Throughput Screening

Based upon the revolutionary Tee-Technology this new DreamFect™ transfection reagent is:

- Highly Efficient
- Simple, ready-to-use & rapid
- Compatible with and without serum-containing culture media
- Multipurpose (various types of nucleic acid)
- Universal (primary cells and cell lines)
- Non toxic & economical

1.2. Kit Contents

OZ Biosciences offers three sizes of DreamFect™ reagents. Kit contents vary according to their size.

- One tube containing 500 L of DreamFect™ good for 125 transfections with 1 g of DNA
- One tube containing 1 mL of DreamFect™ good for 250 transfections with 1 g of DNA
- 5 tubes containing 1 mL of DreamFect™ good for 1250 transfections with 1 g of DNA

Stability and Storage

Storage: +4°C. Upon receipt and for long-term use, store all reagent tubes in the fridge. DreamFect™ kits are stable for at least one year at the recommended storage temperature.

- **DO NOT FREEZE THE DreamFect™ FORMULATION!**
- **DO NOT ADD ANYTHING TO THE STOCK SOLUTION OF DreamFect™ REAGENT!**

Shipping condition: Room Temperature.

2. Applications

2.1. Application Areas

DreamFect™ 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 cells. The DreamFect™ formulation is compatible with serum-containing culture media and serum free culture media. This product is very stable, ready-to-use and intended for research purpose only.

2.2. Cell Types

DreamFect™ reagent can be used with numerous cell types. This technology has been tested successfully on a variety of immortalized cell lines as well as some primary cells. If a particular cell type is not listed in Table 1, this does not imply that DreamFect™ is not going to work. An updated list of cells successfully transfected 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.

Table 1: Example of cells transfected with DreamFect™ reagent.

Cell Lines	***Cell Type***	***Species***	***% Transfected Cells***
A-293	Transformed Kidney	Human	80-90 %
A431	Epidermal Carcinoma	Human	*N.D.*
A549	Non-small cell lung carcinoma	Human	*N.D.*
BHK-21	Kidney	Syrian Hamster	90 %
C2C12	Myoblast	Mouse	*N.D.*
CHO	Ovary (epithelial like)	Chinese Hamster	75-90 %
COS1, COS-7 & Vero	Kidney	Green Monkey	50-70 %
CV-1	Fibroblast-like, Kidney	Green Monkey	*N.D.*
HBL-100	Transformed Breast	Human	40 %
HCT-116	Colon adenocarcinoma	Human	65-70 %
HEK293	Embryonic kidney	Human	90-95%
HeLa	Cervix carcinoma	Human	80 %
HT-22	Hippocampal	Mouse	50-70 %
Hep 3B	Liver Carcinoma	Human	50 %
HepG2	Hepatoma	Human	*N.D.*
LNCaP	Prostate Carcinoma	Human	*N.D.*
MCF-7	Breast Adenocarcinoma	Human	30 %
NIH-3T3	Fibroblasts	Mouse	60-90 %
NS20Y	Neuroblastoma	Mouse	70% *
PC-12	Pheochromocytoma (Adrenal)	Rat	*N.D.*
PT-11	Kidney Fibroblast	Bovine	35%
SW-480	Colon adenocarcinoma	Human	35-45%
Primary Cells			
HDF	Primary Diploid Fibroblasts	Human	70-80 %
MEF	Primary Embryonic Fibroblasts	Mouse	40 %
RHC	Primary Hepatocytes	Wistar Rats	*N.D.*
SMC	Primary smooth muscle	Porcine	*N.D.*

* Transfection complex directly added to fresh seeded cells (see optimization protocol 3.4).

2.3. DreamFect™ and Magnetofection™

DreamFect™ 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™) may have to be adjusted to achieve best results. As a result, we suggest you to optimize the various transfection parameters (components concentration, cell number, incubation time...) as described in section **3.4)** Optimization Protocol. The following recommendations can be used as guidelines to quickly achieve very good transfection. As working concentration, we recommend to use **4µl of DreamFect™ / 1µg of DNA.** DreamFect™ can be used both in the presence and the absence of serum. You can use your routine culture medium for the transfection, except during preparation of the DreamFect™ / 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.
- **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™ 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™ reagent and this effect is cell type dependent and usually small.
- **Materials**. Glass, polypropylene and polystyrene tubes can be used to prepare the DNA and transfection reagent solutions.

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 2). 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.

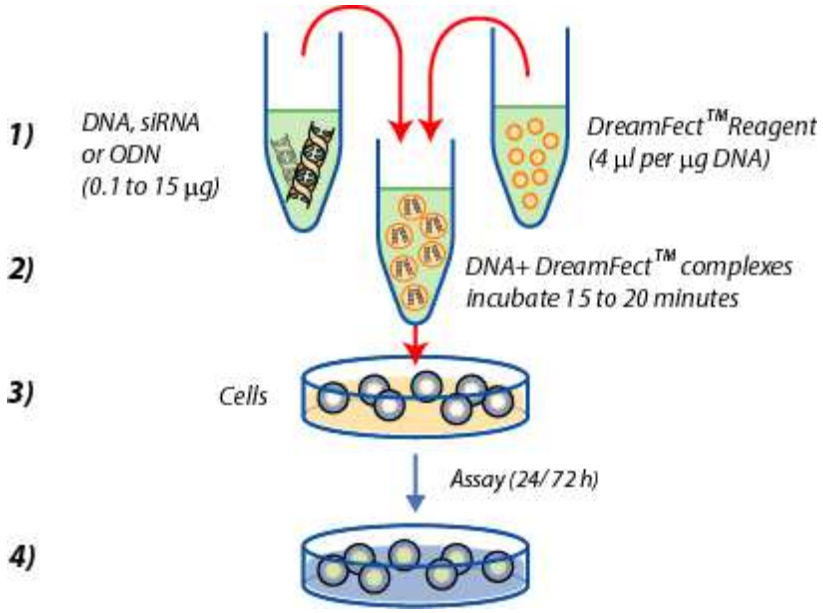
Suspension cells. For fast growing cells, split the cells the day before transfection at a density of 2 to 5 x 10^5 cells / ml, so they are in excellent condition on the day of transfection.

Table 2: Cell number, DNA amount, DreamFect™ volume and transfection conditions suggested.

Tissue Culture Dish	Adherent Cell Number	Suspension Cell Number	DNA Quantity (µg)	DreamFect™ Volume (µl)	Dilution Volume (µl)	Transfection Volume
96 well	0.05 – 0.2 x 10^5	0.5 – 1 x 10^5	0.1	0.4	2 x 25	200 **µl**
24 well	0.5 – 1 x 10^5	1.25 – 5 x 10^5	0.5	2	2 x 50	500 **µl**
12 well	1 – 2 x 10^5	2.5 - 10 x 10^5	1	4	2 x 50	1 mL
6 well	2 – 5 x 10^5	5 – 20 x 10^5	2	8	2 x 100	2 mL
60 mm dish	5 – 10 x 10^5	1 – 5 x 10^6	5	20	2 x 150	4 mL
90 - 100 mm	10 – 30 x 10^5	2.5 – 10 x 10^6	10	40	2 x 250	8 mL
T-75 flask	20 – 50 x 10^5	5 – 15 x 10^6	15	60	2 x 350	10 -12 mL

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.

3.3. Rapid Protocol



The DNA and DreamFect™ 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™ per µg of DNA.

1) . **DNA solution**. Dilute 0.1 to 15 µg of DNA in 25 to 350 µl (see Table 2) of culture medium without serum and antibiotics.
   . **DreamFect™ solution**. Dilute 0.4 to 60 µl of DreamFect™ in 25 to 350 µl (see Table 2) of culture medium without serum and antibiotics.

- **Do not use serum-containing media for this step!**
- **Prevent the DreamFect™ 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™ and DNA stock solution directly into the medium. Contact of DreamFect™ and DNA with the tube surface (plastic or glass) will result in materials lost by adsorption.**

2) Combine the two solutions, mix gently by carefully pipetting up and down and incubate the mixture for 15 - 20 minutes at room temperature. [DreamFect / DNA ratio of 4 can be used as a starting point and ratio might need to be optimized, see below].

- **The diluted solution should be combined within 5 minutes.**
- **Do not vortex or centrifuge!**

3) Add the complexes 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.

- **For transfection of suspension cells, gently mix complexes to the cell solution by pipetting the medium up and down (3-4 times) to ensure a uniform distribution of the mixture. It is important to promote the contact of the complexes with cells during this mixing procedure. In addition, this favors the disruption of potential clumps of cells that are preventing the complexes to get access to all cells.**

4) 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 to72 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. ***
- **If some cytotoxicity is observed in the case of sensitive cells, the medium can be changed after 3-4 hours or 24 hours incubation with fresh medium. ***

* DreamFect / DNA complexes are prepared in medium without serum because serum may interfere with vector assembly. According to the rapid protocol, the serum free complexes cocktail is added to the cells that are covered with complete medium. Therefore, the addition of the transfection cocktail will result 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. In this case, a medium change will be required after the first 4 hours of incubation.

3.4. 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 maximum efficiency in particular cells. For best results, we recommend optimization of the transfection protocol for each combination of plasmid and cell line used. We advise you to optimize your transfection conditions in order to get the best out of DreamFect™. Several parameters can be optimized:

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

OZ Biosciences' team has investigated numerous factors; we recommend that you optimize one parameter at a time and start from the experimental procedure described above (sections 3.1 to 3.3). The two most critical variables are the ratio of DreamFect™ reagent to DNA and the quantity of DNA.

1) DreamFect™ / DNA ratio:

This is an important optimization parameter. DreamFect™ has to be used in 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 ratio of DreamFect™ reagent to DNA over the suggested range in the table 3. You can test ratios from 2 to 12 µl of DreamFect™ reagent per 1 µg DNA.

Table 3: Suggested range of DreamFect™ for optimization.

Tissue Culture Dish	**DNA Quantity** (µg)	DreamFect™ Volume (µL)	DreamFect™ Volume (µL) proposed interval
96 well	0.1	0.2 - 1.2	0.2 –0.4 – 0.6 – 0.8 – 1 – 1.2
24 well	0.5	1 - 6	1 – 2– 3– 4 – 5– 6
12 well	1	2 - 12	2 – 4 – 6 – 8 – 10 – 12
6 well	2	4 - 24	4 – 8 – 12 – 16 – 20 – 24
60 mm dish	5	10 - 60	10 – 20 – 30 – 40 – 50 – 60
90 - 100 mm dish	10	20 - 120	20 – 40 – 60 – 80 – 100 – 120
T-75 flask	15	30 - 180	30 – 60 – 90 – 120 – 150 – 180

2) Quantity of DNA:

In order to obtain the highest transfection efficiency, the amount of DNA used can be increased. However a high amount of the complexes can result in over expression or lysis of the cell. 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™ / DNA ratio proceed to adjust the best amount of DNA required by maintaining a fixed ratio of DreamFect™ reagent to DNA, and vary the DNA quantity over the suggested range (table 4)

Table 3: Suggested range of DNA amounts for optimization.

Tissue Culture Dish	DNA Quantity (µg)	Transfection Volume
96 well	0.05 – 0.4	100 µl
24 well	0.2 – 1	250 µl
12 well	0.4 – 2	500 µl
6 well	0.4 – 5	1 mL
60 mm dish	1 – 12	2 mL
90 - 100 mm dish	2 – 34	5 mL
T-75 flask	2.5 – 42	8 mL

Following these two steps process, culture medium compositions, cell number, incubation times can also be optimized.

3) Cell number:

The cells proliferating rate is also a critical parameter and the optimal confluency have to be adjusted according to the cells used. Thus, the next step is to use the optimize ratio and DNA amount obtained previously and varied 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. Significant gene expression can be detected faster with this method (in 24 hours).

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

4) Effect of serum /Transfection volume:

Almost all cell lines transfected with DreamFect™ 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 medium 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 suggested in Table 1 by those described in Table 3.

5) 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 - 72 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 -galactosidase transgene can be visualized by histochemical staining with X-Gal (see related product).

4. Appendix

4.1 Quality Controls

To assure the performance of each lot of DreamFect™ 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 spots.
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

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.

5. Related Products

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™ (*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)*
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
VeroFect *(for Vero cells)*
FlyFectin *(for Insect cells)*
i-MICST TECHNOLOGY
Viro-MICST (*to transduce directly on magnetic cell purification columns)*
3D TRANSFECTION TECHNOLOGY
3Dfect *(for scaffolds culture)* / 3DfectIN *(for hydrogels culture)*
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit (*for **H**igh **Y**ield **P**rotein **E**xpression)*
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 X-Gal powder 1g / G-418, Sulfate 1g

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

Purchaser Notification

Limited License

The purchase of the DreamFect™ 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 ™ 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 ™ Reagent material and documentation to OZ Biosciences, or by destroying all DreamFect ™ components. Purchasers are advised to contact OZ Biosciences with the notification that a DreamFect ™ 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 ™ 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 ™ 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:

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