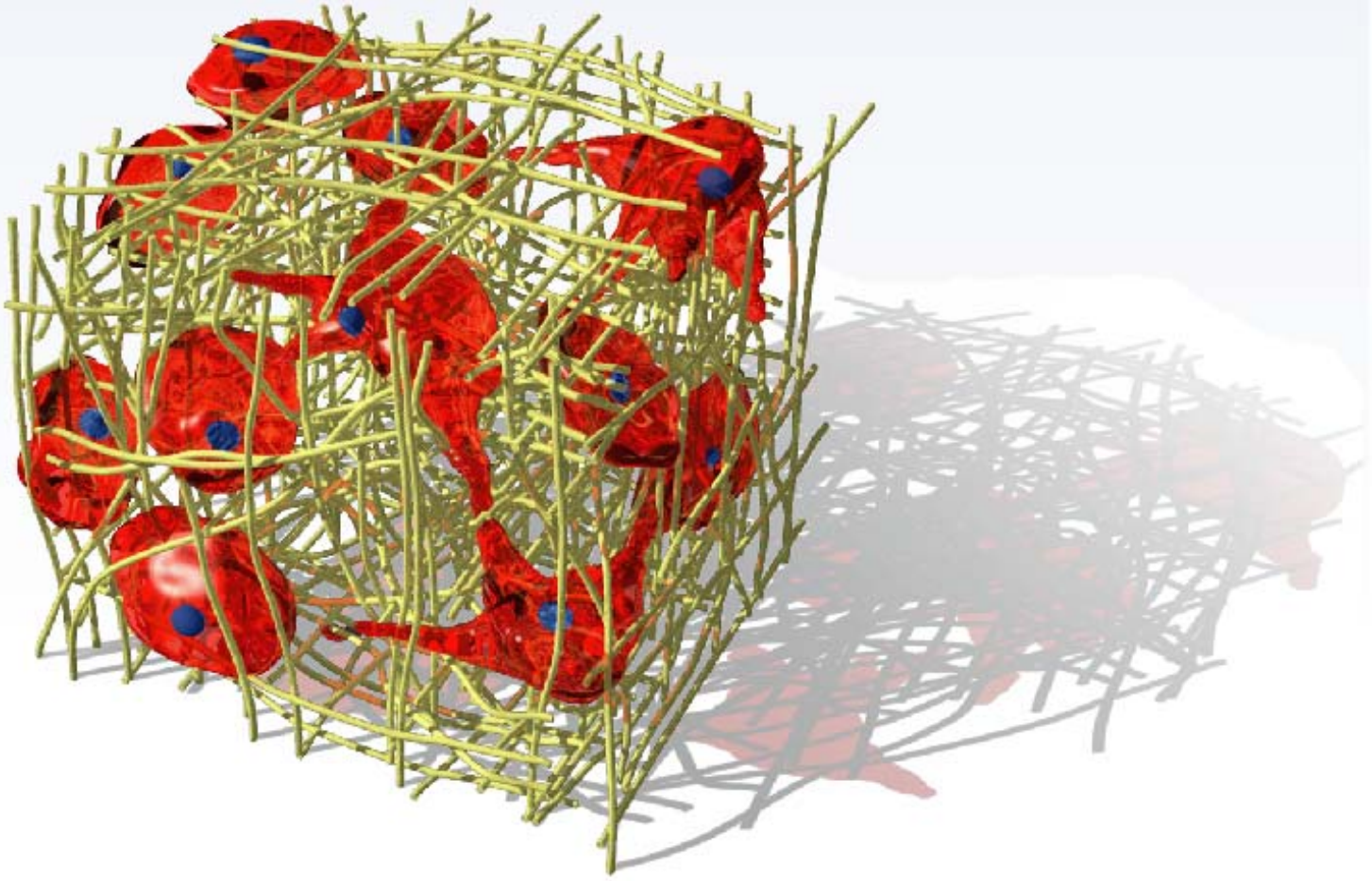
3D-Fect™

INSTRUCTION MANUAL

3D Transfection Reagent for Scaffolds



Sponges, Matrices, Inserts..

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3D-Fect™ transfection reagent

3D Transfection: a new outlook for your cells!!

3D-Fect™ is the latest OZ Biosciences reagent for 3D transfection. It has been specifically designed and developed for transfection of cells cultured in 3D scaffolds (sponges, matrices, inserts…). This reagent is based on a novel technology that allows adding a third dimension to cell cultures.

List of 3D-Fect™ Kits

Catalog Number	Description	Volume (µL)	Size (number of transfection / µg of DNA)
TF20250	3D-Fect™	250	65
TF20500	3D-Fect™	500	125
TF21000	3D-Fect™	1000	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) 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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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

3. General Protocols **3-6**
- 3.1. General Considerations 3
- 3.2. Cell Preparation 3
- 3.3. Protocol for 3D-Scaffold 4
- 3.4. Optimization Protocol 5-6

4. Appendix **7-8**
- 4.1. Quality Controls 7
- 4.2. Troubleshooting 7-8

5. Related Products **9**

6. Purchaser Notification **10**

1. Technology

1.1. Description

Congratulations on your purchase of our latest ***3D-Fect™*** transfection reagent!
3D-Fect™ is our newest transfection reagent specifically designed and developed for cell cultured on 3D Scaffolds. 3D matrices not only add a third dimension to cells' environment, they also allow creating significant differences in cellular characteristics and behavior. Because 3D scaffolds are routinely used in basic research and therapeutic applications, OZ Biosciences has developed two new powerful reagents, ***3D-FectIN ™*** (for hydrogels) *and* ***3D-Fect™*** (for scaffolds). In this way, 3D matrices bearing complexes formed with ***3D-Fect™ reagent*** are colonized by cells to be transfected in a more natural environment. ***3D-Fect™ reagent*** associated with 3D matrices allows numerous cell transfections in order to follow tissue engineering, tissue regeneration, tumor invasion, neural differentiation, cellular polarization, tissue formation, colonization, neurite growth…

Principal **3D-Fect™** advantages:

1. Highly efficient
2. Ideal for any 3D scaffolds (sponges, matrices, insert)
3. Completely biodegradable
4. Universal (primary cells and cell lines)
5. Multipurpose (various types of nucleic acid)
6. Simple, ready-to-use & rapid
7. Serum compatible
8. Appropriate for multiple applications
9. Long term transgene expression

1.2. Kit Content

OZ Biosciences offers two sizes of ***3D-Fect™*** transfection reagent.

- One tube containing 250 µL of 3D-Fect™ good for 65 transfections with 1 µg of DNA
- One tube containing 500 µL of 3D-Fect™ good for 125 transfections with 1 µg of DNA
- One tube containing 1 mL of 3D-Fect™ good for 250 transfections with 1 µg of DNA

Stability and Storage
Storage: Upon reception and for long-term use, store the reagent at 4°C for **3D-Fect™**.

3D-Fect™ is stable for at least one year at +4°C.

Shipping condition: Room temperature.

2. Applications

2.1. Application Areas

3D-Fect™ reagent has been developed for very efficient transfections of nucleic acids into 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 stable, ready-to-use and intended for research purpose only. The field of applications covers tissue engineering, tissue regeneration, tumor invasion, neural differentiation, cellular polarization, tissue formation, colonization, neurite growth, anticancer gene screening, cell survival, growth and differentiation, co-culture…

2.2. Cell Types

3D-Fect™ transfection reagent is suitable for numerous cells. It has been successfully tested on a variety of immortalized and primary cells (see results file). 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.

3. General Protocols

3.1. General Considerations

The instructions given below represent sample protocols that were applied successfully to a variety of cells. Optimal conditions vary depending on the nucleic acid, cell types, scaffolds types and cell culture conditions. Therefore, the amounts and ratio of the individual components (DNA and 3D-Fect™) may have to be adjusted to achieve best results. Accordingly, we suggest you to optimize the various transfection parameters as described in section **3.4)**. The following recommendations can be used as guidelines to quickly achieve very good transfection efficiency. As a starting point, we recommend to use **4 µL of 3D-Fect™ Reagent / 1 µg of DNA.** 3D-Fect™ can be used in the presence or absence of serum. You can use your routine culture medium for the transfection, except during preparation of the 3D-Fect™ / DNA complexes (see **3.3** below).

- **Cells** should be healthy and assayed 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).
- **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 3D-Fect™ 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 3D-Fect™ 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 3D-Fect™ 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

It is recommended to seed the 3D Scaffolds on the day of transfection.
3D scaffolds / sponges. The suitable cell density will depend on the growth rate, the cells conditions and the size of the matrix. In 3D cell culture, the cell number can be increased in comparison to 2D systems. For example, the number of cells may vary from 10,000 cells to more than 100,000 cells for a 0.05 cm^3 3D Scaffold (surface of 0.25 cm^2 x 0.2cm height) (see results file)

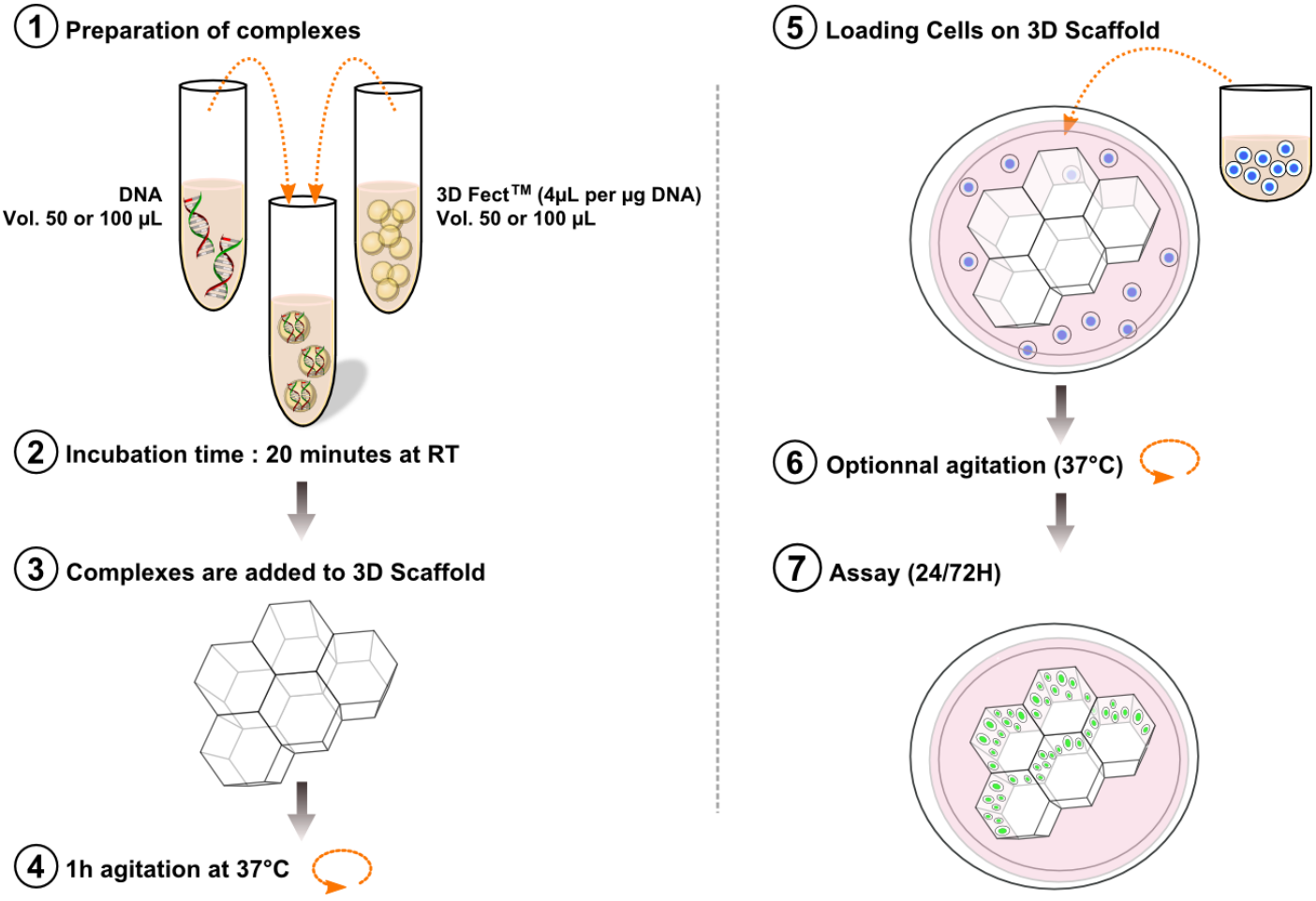
The correct choice of optimal plating density also depends on the planned time between transfection and transgene analysis: for a large interval, prefer lower density and for a short interval a higher density may be advantageous (see section 3.3 for procedure). Optionally, we suggest seeding cells on 3D scaffolds loaded with complexes under slight agitation (150 rpm) from 4 to 24h, to facilitate the matrix colonization.

Table 1: Cell number, DNA amount, 3D-Fect™ volume and transfection conditions suggested for 3D Scaffolds.

Scaffold Size	Adherent Cell Number	DNA (µg)	3D-Fect™ Volume (µL)	Dilution Volume (µL)	Culture Volume
0.05 cm^3 (0.5 x 0.5 x 0.2)	0.1 – 1 x 10^5	1	3 or 4	2 x 50	500 µL
0.125 cm^3 (0.5 x 0.5 x 0.5)	0.25 – 2 x 10^5	3	9 or 12	2 x 50	500 µL
0.5 cm^3 (1 x 1 x 0.5)	1 – 10 x 10^5	15	45 or 60	2 x 100	1 mL

3.3. Protocol for 3D-Scaffolds

Before seeding the cells, matrices must be hydrated with a solution of DNA mixed with 3D-Fect™ reagent for one hour at 37°C. We recommend performing the scaffold re-hydration under slight agitation (150 rpm). For transfection experiments, we advise transferring the hydrated sponge or scaffold to a suitable cell culture dish or well before adding the cells and then incubate under agitation for better colonization.



The DNA and 3D-Fect™ reagent 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 3D-Fect™ per µg of DNA. We suggest beginning with this ratio and optimize it, if required, by following section **3.4.**

Important considerations before beginning transfection:

- 3D-Fect™ reagent must be stored at +4°C.
- Do not use serum-containing media for the preparation of DNA/3D-Fect complexes (step 1)!

- Prevent the 3D-Fect™ reagent 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 3D-Fect™ and DNA stock solution directly into the medium. Contact of 3D-Fect™ and DNA with the tube surface (plastic or glass) could result in materials lost by adsorption.

1) Preparation of DNA/3D-Fect complexes
3D-Fect™ / DNA complexes are prepared in PBS or medium without serum because serum interferes with vector assembly.

- **DNA solution**. Dilute 1 to 15 µg of DNA in 50 or 100 µL of PBS or culture medium without serum and antibiotics as indicated in Table 1. For optimization see section 3.4.

- **3D-Fect™ solution**. Allow the reagent to reach room temperature. Dilute 3 to 60 µL of 3D-Fect™ in 50 or 100 µL of PBS or culture medium without serum and antibiotics as indicated in Table 1. For optimization see section 3.4.

- Add the DNA solution into the 3D-Fect™ solution, mix gently by carefully pipetting up and down 2-3 times. Do not vortex or centrifuge!

2) Incubate the mixture for 20 minutes at room temperature

3) Place the 3D-Scaffold in a suitable cell culture well or dish and add the complexes. Try to avoid bubbles while hydrating the sponge (it can be gently squeezed against the well wall to chase air bubbles).

4) Incubate the hydrated scaffold 1 hour at 37°C. We recommend 150 rpm agitation for better complexes dispersion within the 3D-Scaffold.

5) Transfer the hydrated 3D-Scaffold into an appropriate well or dish and add cells (see Table 1) in complete culture medium.

6) Option: we suggest placing the cells under agitation (150 rpm) at 37°C for 4 to 24h for a better scaffold colonization.

7) 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 from 1 to several days following transfection.

- **For some cells, 24h 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 immediately after cells have colonized the 3D-Scaffold.**

3.4. Optimization Protocol

Although high transfection efficiencies can be achieved in a broad range of cells and scaffolds with the rapid protocol, optimal conditions may vary depending on the nucleic acid, cell type, 3D scaffold composition and complexity, 3D scaffold volume and culture medium composition. Therefore, we recommend optimization of the protocol for each combination of plasmid, cells and scaffold used in order to get the best out of 3D-Fect™ reagent. Consequently, we suggest that you optimized these important parameters:

- Ratio of 3D-Fect™ to DNA
- The quantity of nucleic acid used
- The cell number
- 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 constant. The two most critical variables are the ratio of 3D-Fect™ reagent to DNA and the quantity of DNA.

1) 3D-Fect / DNA ratio:
This is an important optimization parameter. Depending on the 3D matrix, 3D-Fect™ reagent has to be used in slight excess compare to DNA but the optimal ratio will also depend on the cells used. For optimization, first maintain a fixed quantity of DNA (according to the size of your scaffold or cell number) and then vary the amount of 3D-Fect™ reagent over the suggested range in the Table 2. You can test ratios from 1 to 6 µL of 3D-Fect™ reagent per 1 µg DNA.

Table 2: Suggested range of 3D-Fect™ for 3D-Fect™ / DNA ratio optimization.

Scaffold Size	DNA (µg)	3D-Fect™ Volume (µL)	3D-Fect™ Volume (µL) proposed interval
0.05 cm^3 (0.5 x 0.5 x 0.2)	1	1 - 6	1 – 2 – 3 – 4 – 5 – 6
0.125 cm^3 (0.5 x 0.5 x 0.5)	3	3 – 18	3 – 6 – 9 – 12 – 15 - 18
0.5 cm^3 (1 x 1 x 0.5)	15	15 - 90	15 – 30 – 45 – 60 – 75 - 90

2) Quantity of DNA:
After optimization of the 3D-Fect / DNA ratio, proceed to adjust the best amount of DNA by maintaining a fixed ratio of 3D-Fect to DNA, and vary the DNA quantity over the suggested range (Table 3).

Table 3: Suggested range of DNA amounts for optimization with 3D-Fect™.

Scaffold Size	DNA (µg)	DNA quantity (µg) proposed interval
0.05 cm^3 (0.5 x 0.5 x 0.2)	0.5 - 2	0.5 – 1 – 1.5 - 2
0.125 cm^3 (0.5 x 0.5 x 0.5)	1.5 - 6	1.5 – 3 – 4.5 - 6
0.5 cm^3 (1 x 1 x 0.5)	7.5 - 30	7.5 – 15 – 22.5 – 30

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

3) 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.

4) 3D-Fect™ / DNA complex medium:
The buffer or medium composition use to prepare the 3D-Fect / DNA may influence the transfection efficiency. For instance, PBS can be used to prepare the DNA and 3D-Fect™ solutions instead of serum-free medium PBS composition: 137mM NaCl, 2.7mM KCl, 1.5mM KH_2PO_4 and 6.5mM Na_2HPO_4 x 2 H_2O; pH7.4. Other buffers such as HBS, Tris can also be used.

5) Effect of serum /Transfection volume:
Almost all cell lines transfected with 3D-Fect, showed good 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 must be avoided.** Transfection efficiency is delayed since cells have to attach and colonize 3D matrices before transfection can occur. Consequently, the cells may be kept in serum-free or reduced serum conditions during the first 3 to 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.

6) 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 1 to several days. Reporter genes such as GFP, β-galactosidase, secreted alkaline phosphatase or luciferase can be used to quantitatively measured gene expression. These control plasmids completely compatible and successfully tested with 3D-Fect™ reagent are available at www.ozbiosciences.com (pVectOZ- GFP/LacZ/Luc/SEAP/CAT).

OZ Biosciences team has developed a detailed protocol for optimization and also cell specific optimal transfection procedures. 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 3D-Fect™ 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 > 90% of the activity of the reference lot.

4.2. Troubleshooting

Problems	Comments and Suggestions
Low transfection efficiency	1- **3D-Fect™ / nucleic acid ratio.** Optimize the reagent / DNA ratio by using a fixed amount of DNA (µg) and vary the amount of 3D-Fect™ from 4 times less up to 1.5 times more than the suggested amount detailed in the Table 2. 2- **DNA amount.** Use different quantities of DNA with the recommended or optimized (above) 3D-Fect / DNA ratio. 3- **Cell density.** A non-optimal cell density at the time of transfection can lead to insufficient uptake. Optimal cell density is difficult to assess since a third dimension is added in cell culture, try several densities depending on the support. 4- **DNA quality.** DNA should be as pure as possible and free of contaminants (proteins, phenol, ethanol etc.). 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. Other 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 assayed during their exponential growing phase. The presence of contaminants (mycoplasma, fungi) alters considerably the transfection efficiency. 7- **3D matrix colonization**. Ensure that your cells have well colonized the 3D scaffold; to promote or increase colonization, we suggest to perform incubation of the cells and 3D matrix under slight agitation at 37°C. 8- **Medium used for preparing DNA / 3D-Fect 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 3D-Fect™ and DNA solutions with the plastic surface. 9- **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 12h of incubation. 2) The presence of antibiotics might affect cell health and transfection efficiency. 10- **Incubation time and transfection volume.** The optimal time range between transfection and assay varies with cells, promoter, expression product, etc. The transfection efficiency can be monitored after 1 day. Several reporter genes can be used to quantitatively monitored gene expression kinetics. 11- **Old 3D-Fect / DNA complexes.** The 3D-Fect / DNA complexes must be freshly prepared every time. Complexes prepared and stored for longer than 1h can be aggregated. 12- **Transgene detection assay**. Ensure that your post-transfection assay is properly set up

	and includes a positive control. 13- **3D-Fect reagent temperature.** Reagents should have an ambient temperature and be vortexed prior to use. 14- **Transfection reagent storage.** Transfection efficiency can slowly decrease if 3D-Fect™ is kept more than one week at RT. Store at 4°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 conditions (pH, type of medium used, contamination etc), 4) Cells are too confluent or cell density is too low, 5) Verify equipments and materials, 6) ensure compatibility of 3D matrices with cell type. 2- **Matrix Composition.** Ensure that Matrices are compatible with the cells: depending on their compositions, 3D scaffolds will allow cell to attach or not; non adhered cells will go on apoptosis. 3- **Transgene product is toxic.** Use suitable controls such as cells alone, transfection reagent alone or mock transfection with a DNA control. 4- **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. 5- **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. 6- **Incubation time.** Reduce the incubation time of complexes with the cells by replacing the transfection medium by fresh medium after 4h to 24h.

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
3D-Fection Technology	
3D-Fectin (*for all 3D hydrogels : collagen, hyaluronic acid, PEG.*)	TN30500
Magnetofection Technology	
Mega Magnetic Plate	MF14000
Super Magnetic Plate	MF10000
Magnetic Plate 96-magnets	MF10096
PolyMag 1mL *(for all nucleic acids)*	PN31000
PolyMag Neo 1mL (*for all nucleic acids*)	PG61000
LipoMag Kit (*for all nucleic acids*)	LM80500
CombiMag 1mL *(to boost transfection reagent)*	CM21000
SilenceMag 1mL *(for siRNA application)*	SM11000
NeuroMag 1mL *(for transfection of neurons)*	NM51000
ViroMag 1mL (*for all viral applications*)	VM41000
ViroMag R/L 1mL *(for retrovirus and Lentivirus)*	RL41000
AdenoMag 1mL (*for adenovirus*)	AM71000
SelfMag Amino Kit	SA10000
SelfMag Carboxy Kit	SC20000
FluoMag-P 100µL	FP10100
FluoMag-C 100µL	FC10100
FluoMag-S 100µL	FS10100
FluoMag-V 100µL	FV10100
Protein Delivery Systems	
Ab-DeliverIN 1 mL	AI21000
Pro-DeliverIN 1 mL	PI11000
Lipofection Technology (lipid-based)	
Lullaby siRNA transfection reagent 1mL	LL71000
DreamFect Gold Transfection reagent 1mL	DG81000
DreamFect Transfection reagent 1mL	DF41000
EcoTransfect Transfection Reagent 1mL	ET11000
VeroFect Transfection Reagent 1mL	VF61000
FlyFectin Transfection Reagent 1mL	FF51000
CaPO Transfection Kit	CP90000
Plasmids pVectOZ	
pVectOZ-CAT 25µg	PL00010
pVectOZ-GFP 25µg	PL00020
pVectOZ-LacZ 25µg	PL00030
pVectOZ-Luc 25µg	PL00040
pVectOZ-SEAP 25µg	PL00050
Gene & Protein Tools	
Bradford – Protein Assay Kit	BA00100
GeneBlaster selection kit	GB20010
GeneBlaster Emerald	GB20014
β-Galactosidase (ONPG) assay kits	GO10001
β-Galactosidase (CPRG) assay kits	GC10002
X-Gal Staining Kit	GX10003
Biochemical	
D-Luciferin, Na+ 1g	LN10000
D-Luciferin, K+ 1g	LK10000
G-418, Sulfate 1g	GS21000
X-Gal powder 1g	XG11000

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.

Purchaser Notification

Limited License

The purchase of the 3D-Fect™ 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 3D-Fect™ 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 3D-Fect™ Reagent material and documentation to OZ Biosciences, or by destroying all 3D-Fect™ components. Purchasers are advised to contact OZ Biosciences with the notification that a 3D-Fect™ 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 3D-Fect™ 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 3D-Fect™ 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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