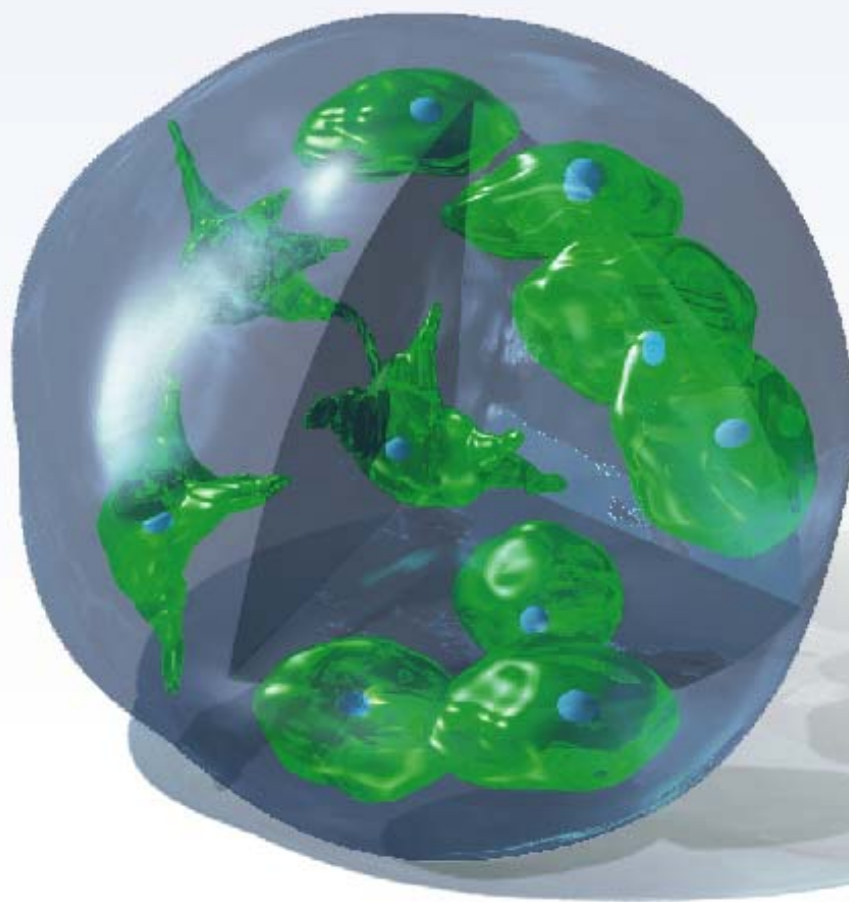


3D-FectIN™
INSTRUCTION MANUAL

**3D Transfection Reagent
for Hydrogels**



**Collagen, Hyaluronic acid, PEG,
Fibrin, Laminin..**



OZ BIOSCIENCES
The art of delivery systems

3D-FectIN™ transfection reagent

3D Transfection: a novel perspective for your cell culture!!

3D-FectIN™ is the latest OZ Biosciences reagent for 3D transfection in hydrogel. It has been specifically designed and developed for transfection of cells cultured in 3D hydrogels (collagen, hyaluronic acid, PEG, laminin...). This reagent is based on a novel technology that allows adding a third dimension to cell cultures.

List of 3D-FectIN™ Kits

Catalog Number	Description	Volume (µL)	Size (number of transfection / µg of DNA)
TN30250	3D-Fectin™	250	65 - 125
TN30500	3D-Fectin™	500	125 - 250
TN31000	3D-Fectin™	1000	250 - 500

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

OZ Biosciences

Parc Scientifique de Luminy
Zone Luminy Entreprise
Case 922
13288 Marseille Cedex 9 - FRANCE.
<http://www.ozbiosciences.com>
Tel: +33 (0)4.86.94.85.16
Fax: +33 (0)4.86.94.85.15
E-mail: contact@ozbiosciences.com
Web Site: www.ozbiosciences.com



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1. Technology

1.1. Description

Congratulations on your purchase of our latest **3D-FectIN™** transfection reagent!

3D-FectIN™ is the newest transfection reagents specifically designed and developed for In-Gel (or hydrogel) 3D cell cultures. 3D matrices not only add a third dimension to cells' environment, they also allow creating significant differences in cellular characteristics and behavior. Because 3D environments are routinely used in basic research and therapeutic applications, OZ Biosciences has developed two new powerful reagents, **3D-FectIN™** (for hydrogel) and **3DFect** (for scaffolds). In this way, 3D matrices (Gels or Hydrogels) bearing complexes formed with **3D-FectIN™ reagent** are colonized by cells to be transfected in a more natural environment. **3D-FectIN™ reagent** associated with 3D matrices allows numerous cell transfections in order to follow angiogenesis, tube and acini formation, colonization, neurite growth, tissue engineering, tissue regeneration, tumor invasion, neural differentiation, cellular polarization, tissue formation...

Principal **3D-Fectin™** advantages:

1. Highly efficient
2. Ideal for any Gel and Hydrogel
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-Fectin™** transfection reagent.

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

Stability and Storage

Storage: Upon reception and for long-term use, store the reagent at -20°C for **3D-FectIN™**.

3D-FectIN™ is stable for at least one year at room temperature, +4°C or -20°C. The storage at -20°C boosts the reagent efficiency and thus is preferred. Nonetheless, the reagent can also be stored at +4°C for shorter period of time. 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

3D-FectIN™ reagent has been developed for 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 very stable, ready-to-use and intended for research purpose only. The field of applications covers angiogenesis, tube and acini formation, colonization, neurite growth, tissue engineering, tissue regeneration, tumor invasion, neural differentiation, wound healing, cellular polarization, tissue formation...

2.2. Cell Types

3D-FectIN™ transfection reagent is suitable for numerous cell types. 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, hydrogel types, and cell culture conditions. Therefore, the amounts and ratio of the individual components (DNA and 3D-FectIN™) 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 **2 to 4 µL of 3D-FectIN™ Reagent / 1 µg of DNA**. 3D-FectIN™ can be used in the presence or in the absence of serum. You can use your routine culture medium for the transfection, except during preparation of the 3D-FectIN / 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-FectIN™ 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 3D-FectIN™ 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-FectIN™ 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 hydrogels on the day of transfection.

3D hydrogels. The suitable cell density will depend on the growth rate, size, ability to invade hydrogels and the cells conditions. Moreover each hydrogel bears specific characteristics regarding the cell type to be used. 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).

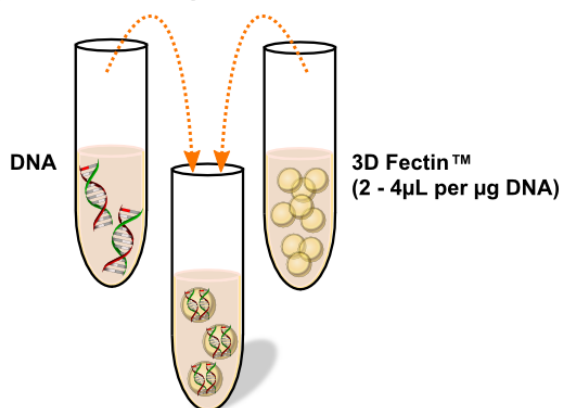
Table 1: Cell number, DNA amount, 3D-FectIN™ volume and transfection conditions suggested for Hydrogels.

Total Hydrogel Volume	Adherent Cell Number	DNA (µg)	3D-Fectin™ Volume (µL)	Dilution Volume (µL)	Hydrogel volume (µL)
50 µL	$0.1 - 0.5 \times 10^5$	2	4 - 8	2 x 12.5	25
100 µL	$0.5 - 2 \times 10^5$	4	8 - 16	2 x 25	50
200 µL	$1 - 4 \times 10^5$	8	16 - 32	2 x 50	100

3.3. Protocol for Hydrogels

Generally, gels must be diluted 50/50 volume with DNA/3D-FectIN complexes. A 50% dilution should be compatible with polymerization capacities of gels and shouldn't interfere with cell growth. Before transfection experiments, we recommend testing cell culture on a 50% gel dilution with medium. Otherwise, we suggest reducing the dilution volume for the preparation of the DNA/3D-FectIN complexes.

1 Preparation of complexes



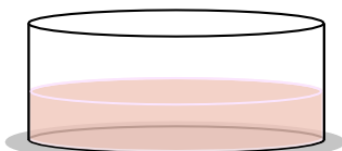
2 Incubation time : 20 minutes at RT



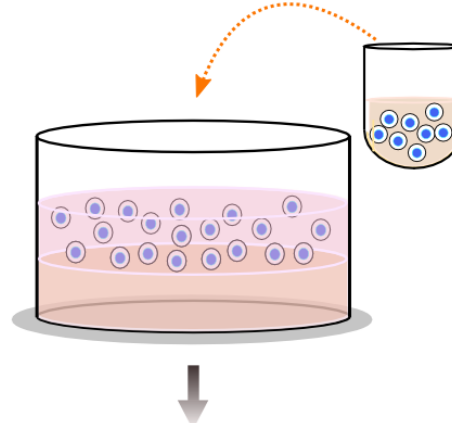
3 Complexes are added to hydrogel



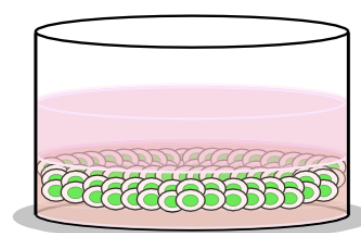
4 Gel polymerization (37°C x 30 min)



5 Loading cells on hydrogel



6 Assay (24/72H)



The DNA and 3D-FectIN™ solutions should have an ambient temperature and be gently vortexed prior to use. The rapid protocol is as simple as follows: Use 2 to 4 µL of 3D-FectIN™ per µg of DNA. We suggest beginning with these ratios (2, 3 and 4) and optimize it, if required, by following section 3.4.

Important considerations before beginning transfection:

- 3D-FectIN™ must be stored at -20°C. If transfections are performed routinely, the transfection reagent can be kept at 4°C for one week after thawing. Thereafter, the reagent should be stored back at -20°C.
- Do not use serum-containing media for the preparation of DNA/3D-Fectin complexes (step 1)!!

- Prevent the 3D-FectIN™ 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-FectIN™ and DNA stock solution directly into the medium. Contact of 3D-FectIN™ and DNA with the tube surface (plastic or glass) will result in materials lost by adsorption.
- For thermo-sensitive gels (i.e. Matrigel™*, BD Biosciences) work on ice with 4°C cooled pipet tips for mixing complexes and gels (step 3) to keep gel in its liquid, non polymerized form for better complexes dispersion.

1) Preparation of DNA/3D-Fectin complexes

3D-Fectin / DNA complexes are prepared in PBS or medium without serum because serum interferes with vector assembly.

- **DNA solution.** Dilute 2 to 8 µg of DNA in 12.5 to 50 µL of PBS or culture medium without serum and antibiotics as indicated in Table 1. For optimization see section 3.4.
- **3D-Fectin™ solution.** Thaw the reagent at room temperature (the reagent can be warm in your hands for faster thawing). Dilute 4 to 32 µL of 3D-Fect™ in 12.5 to 50 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-Fectin™ 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) Mix the complexes with 25 to 100 µL of hydrogel. Avoid bubbles while dispersing complexes within the hydrogel. This step is crucial since complexes dispersion should be done rapidly (to keep liquid hydrogel) while avoiding bubbles that could interfere with transfection.

4) Dispatch these complexes-containing hydrogel in suitable culture dish or well and incubate at 37°C for 30 minutes for gel polymerization.

5) Add cells in complete medium on the gel. Gently rock the plate to homogenize cell suspension.

6) Let the cells colonize the hydrogel and incubate them at 37°C in a CO₂ 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 1 to several days following transfection.

3.4. Optimization Protocol

Although high transfection efficiencies can be achieved in a broad range of cells and hydrogels with the rapid protocol, optimal conditions may vary depending on the nucleic acid, cell type, 3D gel composition and complexity, 3D hydrogel volume and culture medium composition. Therefore, we recommend optimizing the conditions for each combination of plasmid, cells and gel in order to get the best out of 3D-FectIN™ reagent. Several parameters can be optimized:

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

1) 3D-FectIN™ / DNA ratio:

This is an important optimization parameter. Depending on the 3D gel, 3D-FectIN™ reagent has to be used in slight excess compare to DNA but the optimal ratio will also depend on the cells used. For example, in collagen-based gel we suggest the ratio 3D-Fectin / DNA of 4µL/µg DNA whereas for hyaluronic acid-based gel we recommend the ratio of 2/1.

For optimization, first maintain a fixed quantity of DNA (according to the volume of the hydrogel and cell number) and then vary the amount of 3D-FectIN™ over the suggested range in the table 2. You can test ratios from 0.5 to 6 µl of 3D-FectIN™ reagents per 1 µg DNA.

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

Total Hydrogel Volume	DNA (µg)	3D-Fectin™ Volume (µL)	3D-Fectin™ Volume (µL) proposed interval
50 µL	2	2 – 10	2 – 4 – 6 – 8 – 10
100 µL	4	4 – 20	4 – 8 – 12 – 16 – 20
200 µL	8	8 – 40	8 – 16 – 24 – 32 – 40

2) Quantity of DNA:

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

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

Total Hydrogel Volume	DNA (µg)	DNA quantity (µg) proposed interval
50 µL	1-4	1 – 2 – 3 – 4
100 µL	2-8	2 – 4 – 6 – 8
200 µL	4-16	4 – 8 – 12 – 16

Thereafter, culture medium compositions, cell number, 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-FectIN™ / Nucleic acid complex medium:

The buffer or medium composition use to prepare the 3D-Fectin / 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 KH₂PO₄ and 6.5mM Na₂HPO₄ x 2 H₂O; pH7.4. Other buffers such as HBS, Tris can also be used.

5) Effect of serum /Transfection volume:

Almost all cells transfected with 3D-FectIN™, 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 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-FectIN™ reagents 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-Fectin™ 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
<i>Purity</i>	Silica Gel TLC assays. Every compound shall have a single spot.
<i>Sterility</i>	Thioglycolate assay. Absence of fungal and bacterial contamination shall be obtained for 7 days.
<i>Biological Activity</i>	Transfection efficacies on HEK-293 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	<p>1- 3D-FectIN™ / nucleic acid ratio. Optimize the reagent / DNA ratio by using a fixed amount of DNA (µg) and vary the amount of 3D-FectIN™ from 2 times less up to 2.5 times more than the suggested amount detailed in the Table 2.</p> <p>2- Hydrogel / Dilution rate. The protocol is design for a 2X dilution of gel. Try reducing the volume of DNA/3D-FectIN™ mix to change final gel dilution.</p> <p>3- DNA amount. Use different quantity of DNA with the recommended or optimized (above) 3D-Fectin / DNA ratio.</p> <p>4- 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.</p> <p>5- DNA quality. Nucleic acids 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.</p> <p>6- 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.</p> <p>7- 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.</p>
Low transfection efficiency	<p>8- Medium used for preparing DNA / 3D-Fectin 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-FectIN™ and pure DNA solution with the plastic surface.</p> <p>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.</p> <p>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.</p> <p>11- Old 3D-Fectin / DNA complexes. The 3D-Fectin / DNA complexes must be freshly prepared every time. Complexes prepared and stored for longer than 1h can be aggregated.</p>

	<p>12- Transgene detection assay. Ensure that your post-transfection assay is properly set up and includes a positive control.</p> <p>13- 3D-Fectin reagent temperature. Reagents should have an ambient temperature and be vortexed prior to use.</p> <p>14- Transfection reagent storage. Transfection efficiency can slowly decrease if 3D-FectIN™ is kept more than one week at +4°C. Store at -20°C to recover initial efficiency.</p>
Cellular toxicity	<p>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.</p> <p>2- Matrix Composition. Ensure that Matrices are compatible with the cells: depending on their compositions, 3D gel will allow cell to attach or not; non adhered cells can go on apoptosis.</p> <p>3- Transgene product is toxic. Use suitable controls such as cells alone, transfection reagent alone or mock transfection with a DNA control.</p> <p>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.</p> <p>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.</p> <p>6- Incubation time. Reduce the incubation time of complexes with the cells by replacing the transfection medium by fresh medium after 4h to 24h.</p>

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.

* Matrigel™ is a trademark own by BD Biosciences.

Description	Reference
3D-Fection Technology	
3D-Fect (<i>for all 3D scaffolds : sponges, matrices, inserts...</i>)	TF20250
Magnetofection Technology	
Mega Magnetic Plate	MF14000
Super Magnetic Plate	MF10000
Magnetic Plate 96-magnets	MF10096
PolyMag 1mL (<i>for all nucleic acids</i>)	PN31000
PolyMag Neo 1mL (<i>for all nucleic acids</i>)	PG61000
LipoMag Kit (<i>for all nucleic acids</i>)	LM80500
CombiMag 1mL (<i>to boost transfection reagent</i>)	CM21000
SilenceMag 1mL (<i>for siRNA application</i>)	SM11000
NeuroMag 1mL (<i>for transfection of neurons</i>)	NM51000
ViroMag 1mL (<i>for all viral applications</i>)	VM41000
ViroMag R/L 1mL (<i>for retrovirus and Lentivirus</i>)	RL41000
AdenoMag 1mL (<i>for adenovirus</i>)	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>.

Limited License

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

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Product Use Limitations

The 3D-FectIN™ Reagent and all of its components are developed, designed, intended, and sold for research use only. They are not to be used for human diagnostic or included/used in any drug intended for human use. All care and attention should be exercised in the use of the kit components by following proper research laboratory practices.

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

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