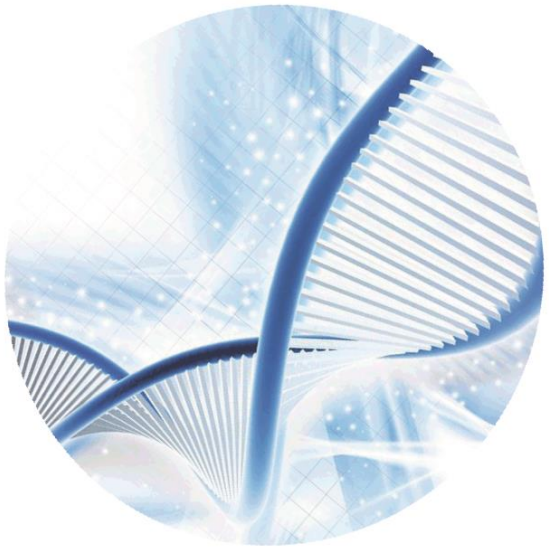


Transfection reagent

3D-Fectin™

3D Transfection Reagent

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

Protocol

We Bring The World Of Biotechnology To You
BOCA SCIENTIFIC

OZBIOSCIENCES
The art of delivery systems

3D-FectIN™ Quick Protocol

To find the ideal conditions, **3D-FectIN™** must be tested at ratios **2 µL/µg** DNA (recommended for hyaluronic acid-based gel) and **4 µL/µg** DNA (recommended for collagen-based gel). For the DNA quantity, we suggest **2 µg** for 50µL of hydrogel, **4 µg** for 100µL of hydrogel and **8 µg** for 200µL of hydrogel.*

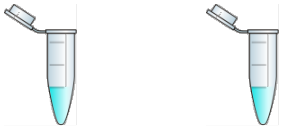
1

Prepare 2 identical tubes of DNA

50 µL of Hydrogel	100 µL of Hydrogel	200 µL of Hydrogel
2µg in 12.5 µL of serum-free medium or buffer* x2	4µg in 25 µL of serum-free medium or buffer* x 2	8µg in 50 µL of serum-free medium or buffer* x 2

2

Prepare 2 tubes of 3D-FectIN™ (with 2 different amounts of reagent*)



50 µL of Hydrogel	100 µL of Hydrogel	200 µL of Hydrogel
4 µL/8 µL in 12.5 µL of serum-free medium or buffer*	8µL/16µL in 25 µL of serum-free medium or buffer*	16 µL/32 µL in 50 µL of serum-free medium or buffer*

3

Mix each tube of DNA (step 1) to each tube of 3D-FectIN™ (step 2) and incubate 20 min at RT

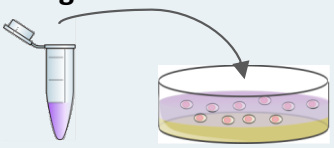
4

Add complexes to hydrogel* and dispatch quickly the mix in suitable culture dish. Incubate at 37°C for 30 min to allow gel polymerization

50 µL of Hydrogel	100 µL of Hydrogel	200 µL of Hydrogel
25µL complexes + 25µL Hydrogel	50µL complexes + 50µL Hydrogel	100µL complexes + 100µL Hydrogel

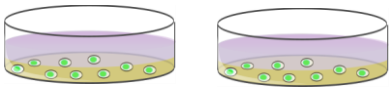
5

Add the cells on the gel and and let them colonize the hydrogel



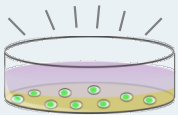
6

Incubate at 37°C until evaluation of transgene expression



7

Choose the best ratio DNA:3D-FectIN™



These conditions might require some further optimizations depending on your cells, scaffolds, DNA, etc.

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ It is recommended to seed the hydrogel the day of transfection.
- ✓ Allow reagents to reach RT and gently vortex them before forming complexes.
- ✓ During preparation of complexes, prevent 3D-FectIN reagent solution to come into contact with any plastic surface that could result in material lost by adsorption. First, add serum-free culture medium to the tube and then mix 3D-FectIN directly into the solution.
- ✓ Medium or buffer without serum & supplement must be used for the DNA/3D-FectIN complexes preparation. Culture medium such as DMEM or OptiMEM or buffers such as HBS or PBS are recommended. In contrast, we do not recommend RPMI for preparing the complexes.
- ✓ In this procedure, gel must be diluted 50/50 volume with DNA/3D-FectIN complexes, be sure that a 50% gel dilution does not interfere with your gel polymerization capacities.
- ✓ For **thermo-sensitive gels** (*i.e.* Matrigel™*, BD Biosciences) work on ice with 4°C cooled pipet tips for mixing complexes and gels to keep gel in its liquid, non-polymerized form for better complexes dispersion.
- ✓ For doses of 3D-FectIN less than 1µL, dilute the reagent with deionized water.

3D-FectIN Reagent | Specifications

Package content	TN30250: 250 µL of 3D-FectIN TN30500: 500 µL of 3D-FectIN TN31000: 1mL of 3D-FectIN
Shipping conditions	Room Temperature
Storage conditions	Store the 3D-FectIN™ transfection reagent at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product Descriptions	3D-FectIN™ is specifically developed to directly transfect cells cultured in 3D hydrogels. 3D-Fect™ is suitable for all kinds of hydrogels and cells
Important notice	For research use only. Not for use in diagnostic procedures

Protocol | DNA or shRNA vectors

1. Cells Preparation

It is recommended to seed the hydrogels on the day of transfection.
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. In 3D cell culture, the cell number can be increased in comparison to 2D systems; please refer to Table 1 below for recommended cell culture conditions.
The correct choice of optimal cell 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 table 1 for suggested number of cells to be seeded).

Total hydrogel volume (µL)	Number of cells	DNA (µg)	3D-FectIN Volume (µL)	Dilution Volume (µL)	Transfection Volume (µL)
50	0.1 - 5 x10^5	2	4 - 8	2 x 12.5	25
100	0.5 - 2 x10^5	4	8 – 16	2 x 25	50
200	1 - 4 x10^5	8	16 - 32	2 x 50	100

Table 1: Suggested transfection conditions

2. Hydrogels preparation

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.

3. DNA/3D-FectIN complexes preparation

a. *3D-FectIN*: Allow the reagent to reach room temperature. Vortex the reagent and dilute the indicated quantity of 3D-FectIN (see Table 1) in 12.5 to 50 µL of culture medium without serum and supplement.

b. *DNA*: Dilute the indicated quantity of DNA (see Table 1) in 12.5 or 50 µL of culture medium without serum and supplement.

c. Add the DNA solution to the 3D-FectIN solutions by carefully pipetting up & down and incubate at room temperature for 20 minutes. Do not vortex or centrifuge

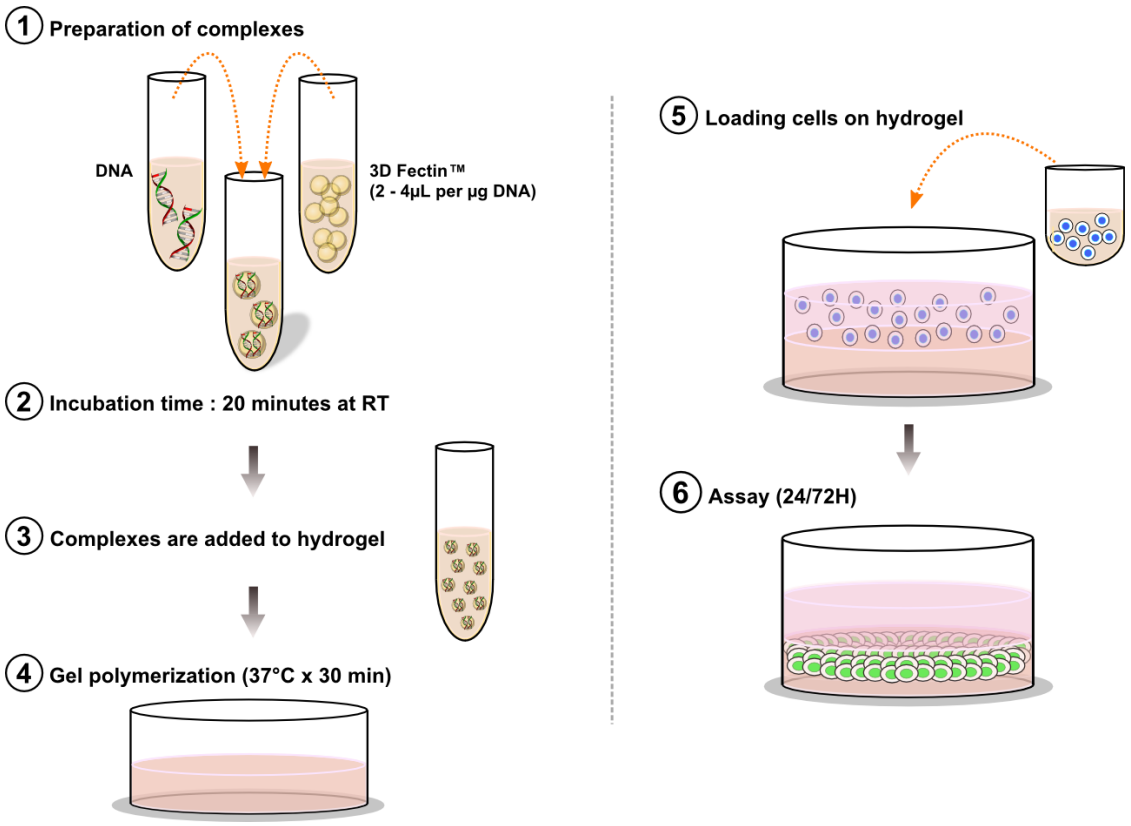
4. Transfection

a. Mix the complexes with 25 to 100 µL of hydrogel (avoid formation of 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.**

b. Dispatch the complexes-containing hydrogel in suitable cell culture dish and incubate at 37°C for 30 min for gel polymerization.

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

d. Let the cells colonize the hydrogel and incubate them 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 1 to several days following transfection).



3DFectIN Protocol steps

NOTES:

- 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 gel.

Optimization Protocol

Optimal conditions may vary depending on the nucleic acid, cell type, 3D gel composition and complexity, 3D hydrogel volume and culture medium composition… We recommend optimizing the following parameters:

- Ratio of 3D-FectIN to DNA and quantity of nucleic acid used
- Cell density
- Culture medium composition (+/- serum) and reagent / nucleic acid complex medium
- Incubation time

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

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.

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

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

2. Quantity of DNA

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 (refer to Table 3).

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

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

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. Use the transfection conditions found 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 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

Transfection efficiency can be increased without serum or under reduced serum condition. Consequently, the cells may be kept in serum-free or reduced serum conditions during the first 3 to 4 hours of transfection. Transfection efficiency is delayed since cells have to colonize 3D matrices before transfection can occur. 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. **Remember that presence of serum during complex formation must be avoided.**

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.

Additional products for your 3D transfection experiments

- **3DFect** for transfection in 3D scaffolds
- **si3DFectIN** for siRNA transfection in 3D hydrogels

Purchaser Notification

Limited License

The purchase of this product 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 purposes 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 this product. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, please contact us. Buyers may end this License at any time by returning the product and documentation to OZ Biosciences, or by destroying all components. Purchasers are advised to contact us with the notification that the product 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 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

This product 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.

We Bring The World Of Biotechnology To You

BOCA SCIENTIFIC

www.bocascientific.com
(781) 686-1631
info@bocascientific.com

Rev. 01/2018