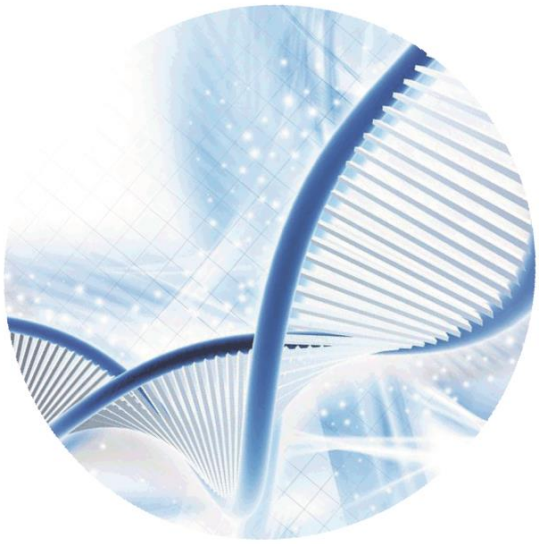


Transfection reagent

3D-Fectin™

3D Transfection Reagent

siRNA Delivery into Hydrogels (Collagen, Hyaluronic acid, PEG, Fibrin...)

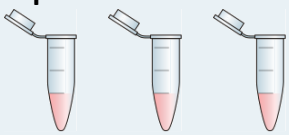
Protocol

OZBIOSCIENCES
The art of delivery systems

3D-FectIN™ Quick Protocol

To find the ideal conditions for gene silencing with 3D-FectIN, we suggest to test increasing doses of **3D-FectIN™** with a fixed concentration, 50nM of siRNA

1 **Prepare 3 identical tubes of siRNA**



50 µL of Hydrogel	100 µL of Hydrogel	200 µL of Hydrogel
50 nM in 12.5 µL of serum-free medium or buffer* x3	50 nM in 25 µL of serum-free medium or buffer* x 3	50nM in 50 µL of serum-free medium or buffer* x 3

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

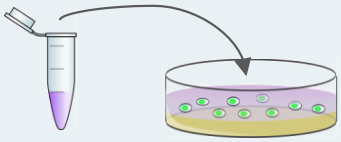
50 µL of Hydrogel	100 µL of Hydrogel	200 µL of Hydrogel
4 µL/6µL/8 µL in 12.5 µL of serum-free medium or buffer*	8µL/12µL/16µL in 25 µL of serum-free medium or buffer*	16 µL/24µL/32 µL in 50 µL of serum-free medium or buffer*

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

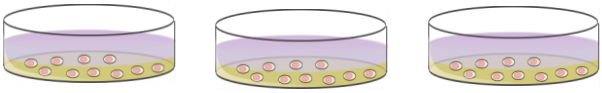
4 **Add complexes to hydrogel and dispatch quickly the mix in suitable culture dish. Incubate at 37°C for 30 min to allow gel polymerization**

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

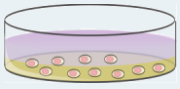
5 **Add the cells on the gel and and let them colonize the hydrogel**



6 **Incubate at 37°C until evaluation of transgene expression**



7 **Choose the best ratio siRNA:3D-FectIN™**



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

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

www.ozbiosciences.com

Any questions?

tech@ozbiosciences.com

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 description	3D-FectIN™ reagent is specifically designed and developed for silencing gene expression in cells cultured in gels (or hydrogels).
Important notice	For research use only. Not for use in diagnostic procedures

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 optimal plating density also depends on the planned time between transfection and gene silencing analysis: for a large interval, prefer lower density as gene silencing generally occurs later than gene expression.

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. siRNA/3D-FectIN complexes preparation

a. *3D-FectIN*: Vortex the reagent and dilute the indicated quantity of 3D-FectIN (refer to table 1) in 12.5 to 50 µL of culture medium without serum and supplement.

b. *siRNA*: Dilute the indicated quantity of siRNA (see Table 1) in 12.5 or 50 µL of culture medium without serum and supplement for a final concentration of 50 nM.

c. Add the siRNA solution to the 3D-FectIN solutions by carefully pipetting up & down and incubate at room temperature for 20 minutes.

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

Table 1: Transfection conditions suggested

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**

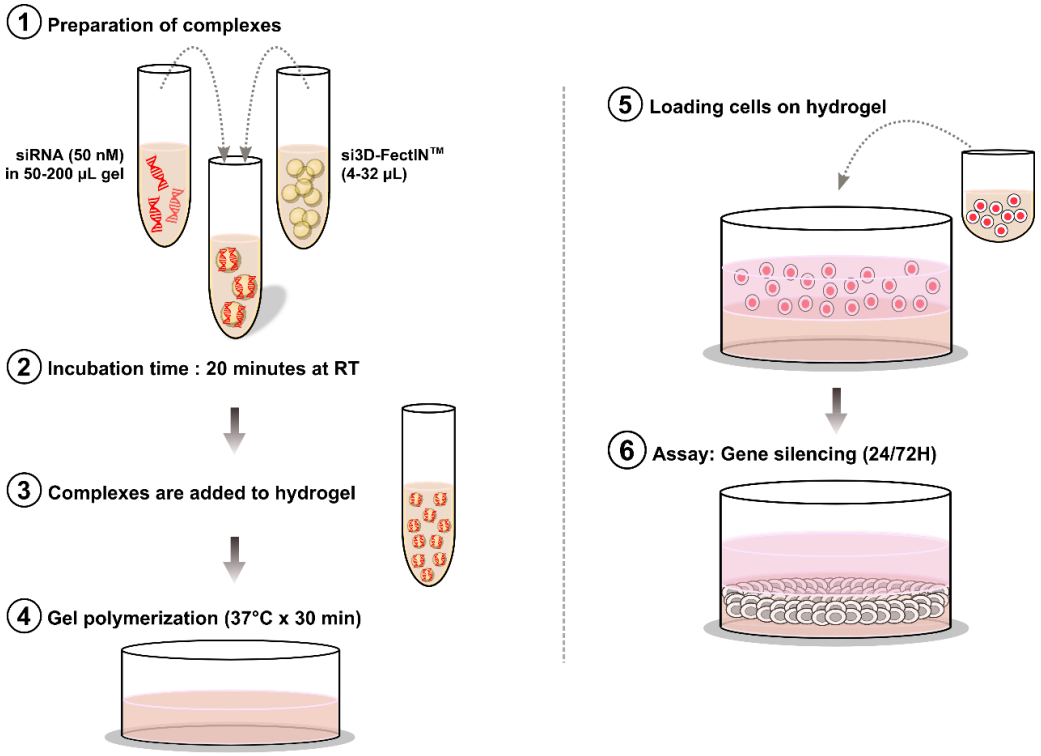
b. Dispatch these complexes-containing hydrogels in suitable cell culture dish and incubate at 37°C for 30 min for gel polymerization.

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 gene silencing.

5. Medium Change

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.



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

1. Ratio of 3D-FectIN™ to siRNA

Maintain a fixed quantity of siRNA (according to the size of your gel or cell number) and then vary the amount of 3D-FectIN™ reagent over the suggested range in the Table 2 below.

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

Table 2: Suggested range of 3D-FectIN™ for optimization using 50 nM siRNA

2. The quantity of nucleic acid used

Once the optimal 3D-FectIN™ / siRNA ratio is found, adjust the siRNA concentration according to Table 3.

Total Hydrogel Volume	siRNA (nM)	siRNA concentration (nM) proposed interval
50 µL	25 - 200	25 – 50 – 100 - 200
100 µL		
200 µL		

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

Finally, culture medium composition (for preparing the complexes), cell density, total culture medium volume and incubation time can also be optimized.
We recommend you to 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 siRNA and the concentration of siRNA.

Additional products for your 3D transfection experiments

- **3DFect** for transfection in 3D scaffolds
- **3DFect** for siRNA transfection in 3D scaffolds
- **3DFectIN** for DNA transfection in 3D hydrogels

Purchaser Notification

Limited License
The purchase of the 3D-FectIN kit 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 kit. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact us at OZ Biosciences. Buyers may end this License at any time by returning all 3D-FectIN kit reagents 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). This document covers entirely the terms of the 3D-FectIN kit 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
3D-FectIN kit 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.

EUROPE & ASIA
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

USA & CANADA
OZ Biosciences INC

4901 Morena Blvd
Suite 901
San Diego CA 92117
USA

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

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

Rev. 06/2019