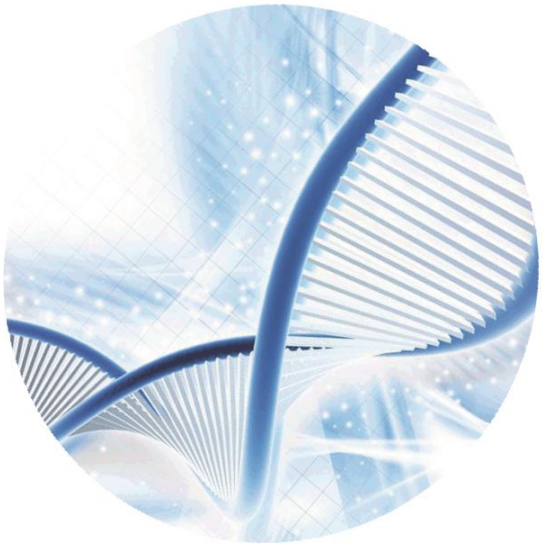


Transfection reagent

si3D-Fect™

3D Transfection Reagent for Scaffolds
Dedicated to siRNA Delivery

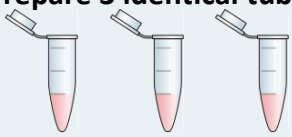
Protocol

OZBIOSCIENCES
The art of delivery systems

si3D-Fect™ Quick Protocol

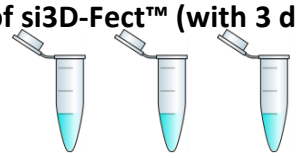
To find the ideal conditions for gene silencing with si3D-Fect, we suggest to test increasing doses of **si3D-Fect™** with a fixed concentration of siRNA: 50nM

1 — Prepare 3 identical tubes of siRNA



0.05 cm³ scaffold	0.125 cm³ scaffold	0.5 cm³ scaffold
50nM in 50µL of serum-free medium or buffer* x3	50nM in 100µL of serum-free medium or buffer* x3	50nM in 200µL of serum-free medium or buffer* x3

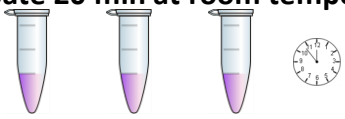
2 — Prepare 3 tubes of si3D-Fect™ (with 3 different amounts of reagent)



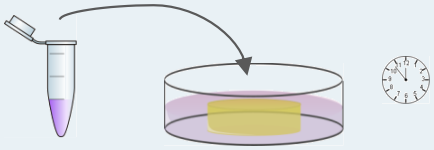
0.05 cm³ scaffold	0.125 cm³ scaffold	0.5 cm³ scaffold
4µL/6µL /8µL in 50µL of serum-free medium or buffer*	12µL/18µL/24µL in 100µL of serum-free medium or buffer*	24µL/36µL/48µL in 200µL of serum-free medium or buffer*

3 — Mix each tube of siRNA (step 1) to each tube of si3D-Fect™ (step 2)

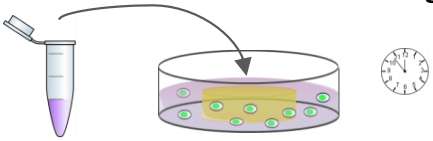
4 — Incubate 20 min at room temperature



5 — Distribute each mix onto the 3D Scaffold and incubate 1h under agitation at 37°C

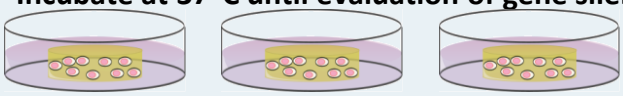


56 — Add cells to the 3D scaffold and incubate under agitation at 37°C for 4 to 24h

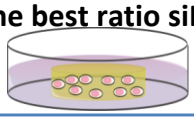


0.05 cm³ scaffold	0.125 cm³ scaffold	0.5 cm³ scaffold
0.1 – 1 x 10^5 cells	0.25 – 2 x 10^5 cells	1 – 10 x 10^5 cells

7 — Incubate at 37°C until evaluation of gene silencing



8 — Choose the best ratio siRNA : si3D-Fect™



* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ It is recommended to seed the 3D-scaffold the day of transfection.
- ✓ Allow reagents to reach RT and gently vortex them before forming complexes.
- ✓ During preparation of complexes, prevent 3D-Fect 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/si3D-Fect 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.
- ✓ For doses of si3D-Fect 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	STF40250: 250 µL of si3D-Fect STF40500: 500 µL of si3D-Fect STF41000: 1mL of si3D-Fect
Shipping conditions	Room Temperature
Storage conditions	Store the si3D-Fect™ transfection reagent at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product description	si3D-Fect™ reagent is specifically designed and developed for silencing gene expression in cells cultured in 3D Scaffolds.
Important notice	For research use only. Not for use in diagnostic procedures

1. Cells preparation

It is recommended to seed the 3D Scaffolds on the day of transfection.
The suitable cell density will depend on their 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; 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.
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.

Scaffold size (cm^3)	Number of cells	siRNA (nM)	si3D-Fect Volume (µL)	Dilution Volume (µL)	Transfection Volume (µL)
0.05	0.1 - 1 $x10^5$	50	4- 8	2 x 50	500 µL
0.125	0.25 - 2 $x10^5$	50	12 - 24	2 x 50	500 µL
0.5	1 - 10 $x10^5$	50	24 - 48	2 x 100	1 mL

Table 1: Suggested transfection conditions

2. Scaffold preparation

Before seeding the cells, matrices must be hydrated with a solution of DNA mixed with si3D-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.

3. siRNA/si3D-Fect complexes preparation

a. *si3D-Fect*: Vortex the reagent and dilute the indicated quantity of si3D-Fect (refer to table 1) in 50 to 100 µL of culture medium without serum and supplement.

b. *siRNA*: Dilute the indicated quantity of siRNA (see Table 1) in 50 or 100 µL of culture medium without serum and supplement for a final concentration of 50 nM.

c. Add the siRNA solution to the si3D-Fect solutions by carefully pipetting up & down and incubate at room temperature for 20 minutes.gfd

4. Transfection

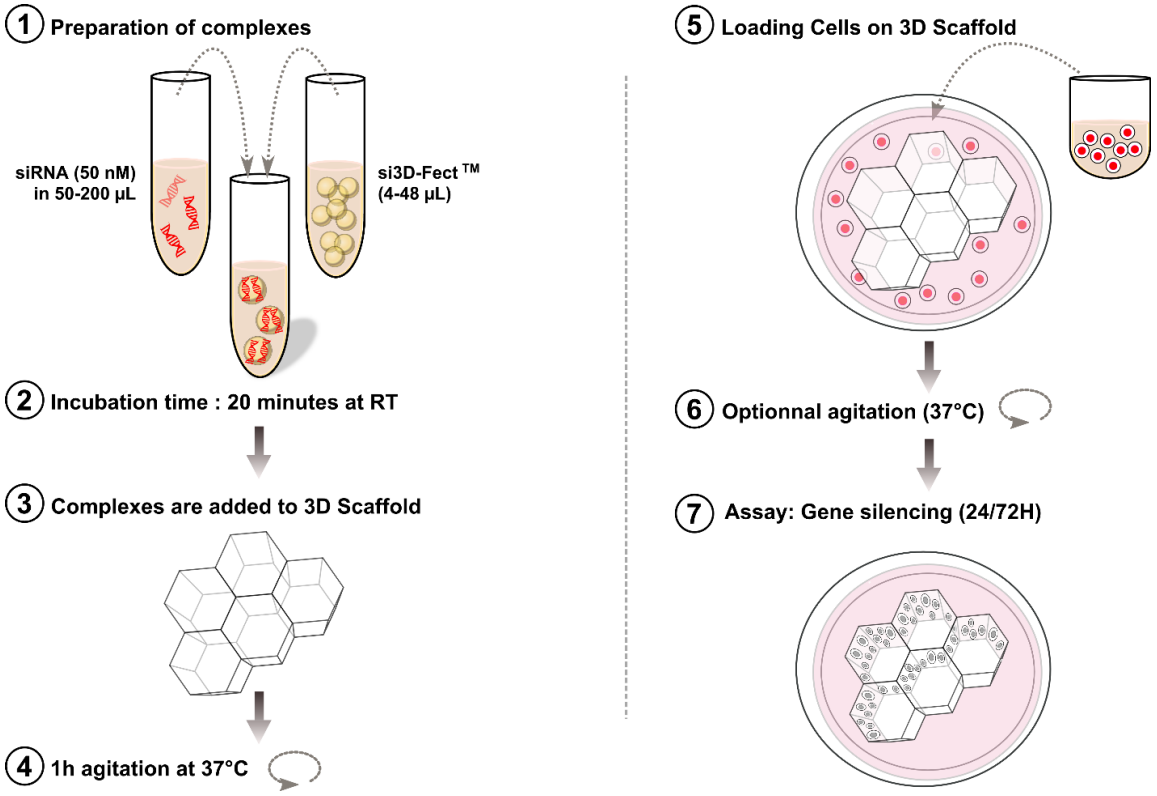
a. Place the 3D-Scaffold in a suitable cell culture dish and add the si3D-Fect / siRNA complexes drop by drop. Try to avoid bubbles while hydrating the sponge (it can be gently squeezed against the well wall to chase air bubbles)..

b. Incubate the hydrated scaffold 1h at 37°C under agitation (150 rpm) for better complexes dispersion within the 3D-Scaffold.

c. Transfer the hydrated 3D-scaffold into an appropriate cell culture dish and add cells in complete culture medium (refer to table 1).

NOTE: cells can be placed under agitation (150 rpm) for a better scaffold colonization.

d. Incubate the cells at 37°C in a CO_2 incubator under standard conditions until evaluation of gene silencing.



IMPORTANT CONSIDERATIONS

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.

Optimization Protocol

To achieve the highest transfection efficiency, the following parameters can be optimized:

1. Ratio of si3D-Fect™ to siRNA

Maintain a fixed quantity of siRNA (according to the size of your scaffold or cell number) and then vary the amount of si3D-Fect™ reagent over the suggested range in the Table 2 below.

Scaffold Size	si3D-Fect™ Volume (µL)	si3D-Fect™ Volume (µL) proposed interval
0.05 cm^3 (0.5 x 0.5 x 0.2)	4 - 12	4 – 6 – 8 – 12
0.125 cm^3 (0.5 x 0.5 x 0.5)	12 – 36	12 – 18 – 24 – 36
0.5 cm^3 (1 x 1 x 0.5)	24 - 96	24 – 36 – 48 –96

Table 2: Suggested range of si3D-Fect™ for optimization using 50 nM siRNA

2. The quantity of nucleic acid used

Once the optimal si3D-Fect™ / siRNA ratio is found, adjust the siRNA according to Table 3.

Scaffold Size	siRNA (nM)	siRNA concentration (nM) proposed interval
0.05 cm^3 (0.5 x 0.5 x 0.2)	25 - 200	25 – 50 – 100 - 200
0.125 cm^3 (0.5 x 0.5 x 0.5)		
0.5 cm^3 (1 x 1 x 0.5)		

Table 3: Suggested range of siRNA amounts for optimization with si3D-Fect™

Finally, culture medium compositions (for preparing the complexes), cell density, total culture medium volume and incubation times 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 si3D-Fect™ reagent to siRNA and the amount of siRNA.

Additional products for your 3D transfection experiments:

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

Purchaser Notification

Limited License
The purchase of the si3D-Fect 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 si3D-Fect 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 si3D-Fect kit reagents and documentation to OZ Biosciences, or by destroying all si3D-Fect components. Purchasers are advised to contact OZ Biosciences with the notification that a si3D-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 si3D-Fect 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
si3D-Fect 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.

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