Transfection reagent

3D-Fect™

3D Transfection Reagent for Scaffolds

Sponges, Matrices, Inserts...

Protocol

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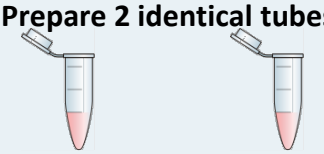
3D-Fect™ Quick Protocol

To find the ideal conditions, **3D-Fect™** must be tested at ratios **3 µL/µg** DNA and **4 µL/µg** DNA. For the DNA quantity, we suggest **1 µg** per 0.05 cm^3 scaffold, **3 µg** per 0.125 cm^3 scaffold and **15 µg** per 0.5 cm^3 scaffold.*

1 — Prepare 2 identical tubes of DNA



0.05 cm^3 scaffold	0.125 cm^3 scaffold	0.5 cm^3 scaffold
1µg in 50µL of serum-free medium or buffer* x2	3µg in 50µL of serum-free medium or buffer* x 2	15µg in 100µL of serum-free medium or buffer* x 2

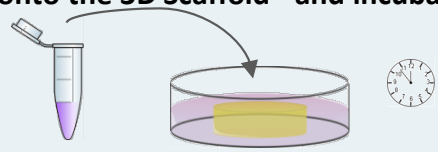
2 — Prepare 2 tubes of 3D-Fect™ (with 2 different amounts of reagent*)

0.05 cm^3 scaffold	0.125 cm^3 scaffold	0.5 cm^3 scaffold
3µL/4µL in 50µL of serum-free medium or buffer*	9µL/12µL in 50µL of serum-free medium or buffer*	45µL/60µL in 100µL of serum-free medium or buffer*

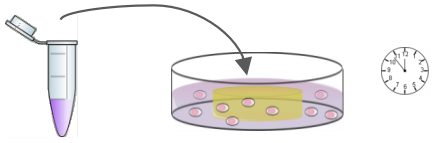
3 — Mix each tube of DNA (step 1) to each tube of 3D-Fect™ (step 2)

4 — Incubate 20 min at room temperature

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



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



0.05 cm^3 scaffold	0.125 cm^3 scaffold	0.5 cm^3 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 transgene expression

8 — Choose the best ratio DNA:3D-Fect™

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 <u>without serum & supplement</u>** must be used for the DNA/3D-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 3D-Fect less than 1µL, dilute the reagent with deionized water.

3D-Fect Reagent | Specifications

Package content	TF20250: 250 µL of 3D-Fect TF20500: 500 µL of 3D-Fect TF21000: 1mL of 3D-Fect
Shipping conditions	Room Temperature
Storage conditions	Store the 3D-Fect™ transfection reagent at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product Descriptions	3D-Fect™ is specifically developed to directly transfect cells cultured in 3D scaffolds. 3D-Fect™ is suitable for all kinds of scaffolds and cells.
Important notice	For research use only. Not for use in diagnostic procedures

1. Cell Preparation

It is recommended to seed the 3D Scaffolds on the day of transfection.
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).
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 (refer to Table 1). 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	DNA (µg)	3D-Fect Volume (µL)	Dilution Volume (µL)	Transfection Volume
0.05 (0.5 x 0.5 x 0.2)	0.1 - 1 x10^5	1	3 - 4	2 x 50	500 µL
0.125 (0.5 x 0.5 x 0.5)	0.25 - 2 x10^5	3	9 - 12	2 x 50	500 µL
0.5 (1 x 1 x 0.5)	1 - 10 x10^5	15	45 - 60	2 x 100	1 mL

Table 1: Recommended starting conditions

2. Scaffold preparation

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.

3. DNA/3D-Fect complexes preparation

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

a. *3D-Fect*: Allow the reagent to reach Room Temperature. Vortex the reagent and dilute the indicated quantity of 3D-Fect (refer to Table 1) in 50 to 100 µL of culture medium without serum and supplement. For optimization see next section.

b. *DNA*: Dilute the indicated quantity of DNA (refer to Table 1) in 50 or 100 µL of culture medium without serum and supplement.

c. Add the DNA solution to the 3D-Fect solutions, mix gently by carefully pipetting up & down and incubate at room temperature for 20 minutes.

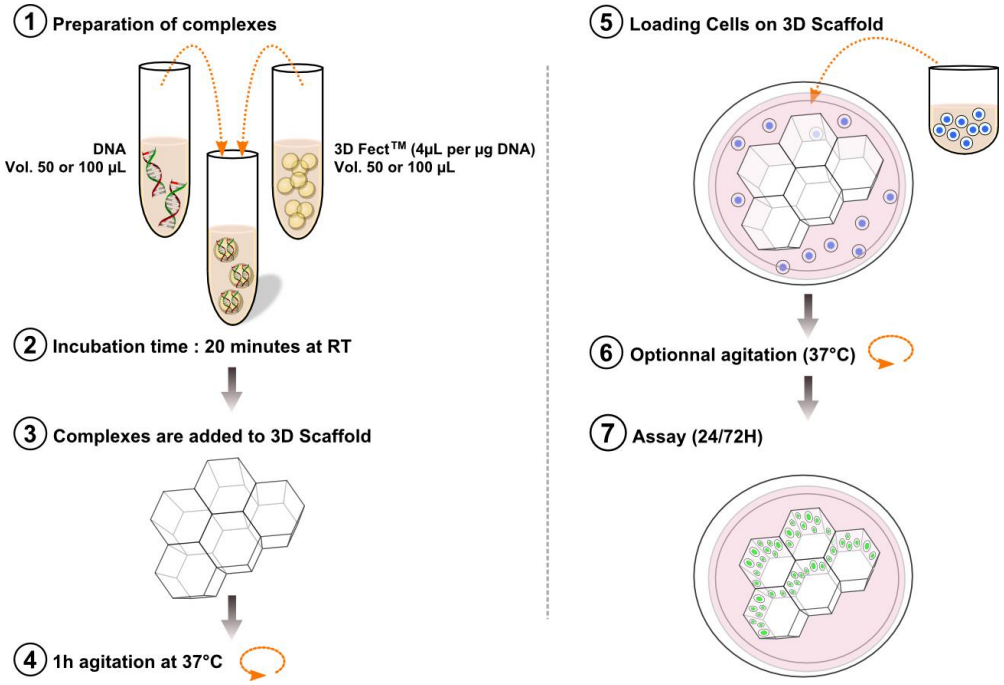
4. Transfection

a. Place the 3D-Scaffold in a suitable cell culture dish and add the 3D-Fect / DNA 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. We recommend 150 rpm agitation 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 transgene expression.



3DFect Protocol steps

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

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

- Ratio of 3D-Fect™ to DNA and quantity of nucleic acid used
- Cell number
- 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-Fect™ reagent to DNA and the quantity of DNA.

1. 3D-Fect / DNA ratio

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.

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

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

2. Quantity of DNA

Adjust the amount of DNA by maintaining a fixed ratio of 3D-Fect to DNA, and vary the DNA quantity over the suggested range (Table 3).

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

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

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 recommended starting conditions (see Table 1) and vary the cell number to be assayed.

4. 3D-Fect™ / DNA complex medium

The buffer or medium composition used 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

Transfection efficiency can be increased in absence of serum or under reduced serum condition. Consequently, 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.

Reporter genes such as GFP, β-galactosidase, secreted alkaline phosphatase or luciferase can be used to quantitatively measured gene expression.

Additional products for your 3D transfection experiments:

- **3DFectIN transfection reagent** for transfection in 3D hydrogels
- **si3DFect transfection reagent** for siRNA transfection in 3D scaffolds
- **pVectOZ Transfection plasmids (CAT, GFP, LacZ, Luciferase, SEAP)** - Positive controls and optimization of all transfection experiments

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