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

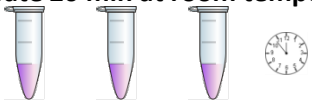
0.05 cm³ scaffold	0.125 cm³ scaffold	0.5 cm³ scaffold
50nM in 50µL of serum-free medium or buffer* x3	50nM in 100µL of serum-free medium or buffer* x3	50nM in 200µL of serum-free medium or buffer* x3

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

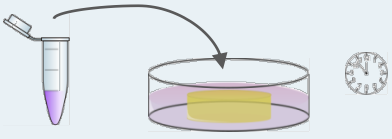
0.05 cm³ scaffold	0.125 cm³ scaffold	0.5 cm³ scaffold
4µL/6µL /8µL in 50µL of serum-free medium or buffer*	12µL/18µL/24µL in 100µL of serum-free medium or buffer*	24µL/36µL/48µL in 200µL of serum-free medium or buffer*

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

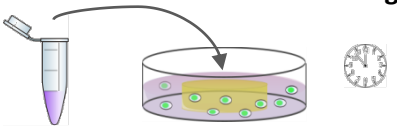
4 — Incubate 20 min at room temperature



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

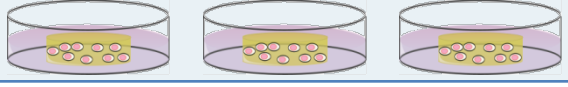


56 — Add cells to the 3D scaffold and incubate under agitation at 37°C for 4 to 24h

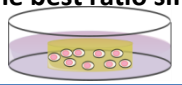


0.05 cm³ scaffold	0.125 cm³ scaffold	0.5 cm³ scaffold
0.1 – 1 x 10^5 cells	0.25 – 2 x 10^5 cells	1 – 10 x 10^5 cells

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



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



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***NOTES:**

(1) Of course the conditions provided above might required some further optimizations depending on your cells, scaffolds, DNA etc…

(2) It is recommended to seed the 3D-scaffold the day of transfection.

(3) Allow reagents to reach RT and gently vortex them before forming complexes .

(4) Medium or buffer <u>without serum & supplement</u> 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.

(5) For doses of si3D-Fect less than 1µL, dilute the reagent with deionized water.

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