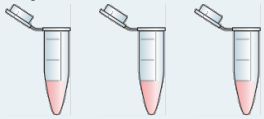
si3D-FectIN™ Quick Protocol

To find the ideal conditions for gene silencing with si3D-FectIN, we suggest to test increasing doses of **si3D-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 si3D-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 si3D-FectIN™ (step 2) & incubate 20 min at RT

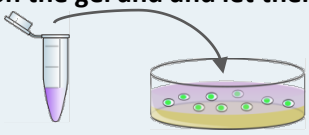
4

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

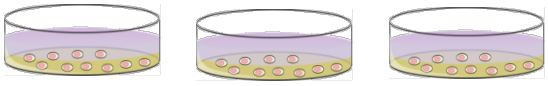
5

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



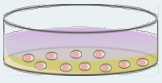
6

Incubate at 37°C until evaluation of transgene expression



7

Choose the best ratio siRNA:si3D-FectIN™



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***NOTES:**

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

(2) It is recommended to seed the hydrogel 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-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.

(5) In this procedure, gel must be diluted 50/50 volume with DNA/si3D-FectIN complexes, be sure that a 50% gel dilution does not interfere with your gel polymerization capacities.

(6) For doses of 3D-Fect less than 1µL, dilute the reagent with deionized water.

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www.bocascientific.com

(781) 686-1631

info@bocascientific.com