FlyFectin™ Quick Protocol

transfection/co-transfection

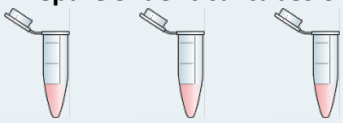
To find the ideal conditions, FlyFectin™ must be tested at ratios **3 µL/µg**, **5 µL/µg** and **7 µL/µg** (µL of FlyFectin / µg of DNA). For the DNA quantity, we suggest 1 **µg** per well in 24-well and **2 µg** per well in 6-well. For co-transfection, divide the total DNA amount into a mix of the different plasmids needed.

1

One to 4 hours (suspension cells) or 18 to 24h (adherent cells) before transfection, seed cells to be at 60-80% confluency

2

Prepare 3 identical tubes of DNA



24 well plate	6 well plate
1µg in 50µL of serum-free medium or buffer* x 3	2µg in 100µL of serum-free medium or buffer* x 3

3

Prepare 3 tubes of FlyFectin™ (with 3 different amounts of reagent)

24 well plate	6 well plate
3µL/5µL/7µL in 50µL of serum-free medium or buffer*	6µL/10µL/14µL in 100µL of serum-free medium or buffer*

4

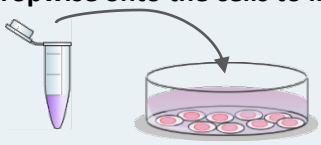
Mix each tube of DNA (step 2) to each tube of FlyFectin™ (step 3)

5

Incubate 20 min at room temperature

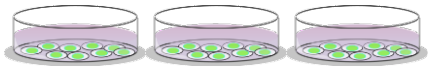
6

Distribute each mix dropwise onto the cells to insure uniform distribution



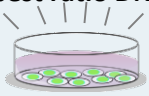
7

Incubate cells for 24 to 72h at 37°C until evaluation of transgene expression



8

Choose the best ratio DNA:FlyFectin™



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***NOTES:**

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

(2) For cell lines, 24h before transfection seed the cells in a 96-well plate, 24-well plate or 6-well plate in respectively 150 µL, 400 µL and 2 mL of complete culture medium.
Cells should be healthy and assay during their exponential growing phase. The cells proliferating rate is a critical parameter and the optimal confluency has to be adjusted according to the cells used. Do not use cells cultured longer than 4 months. Maintain plates at 27°C in an atmosphere free of CO2.

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

(4) **Medium or buffer without serum & supplement** must be used for the DNA/Flyfectin complexes preparation. Culture medium such as Insect media, MEM, 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 Flyfectin less than 1µL, dilute the reagent with deionized water.

(6) For some cells, 24 hours 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 after 3-4 hours.

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www.bocascientific.com
(781) 686-1631
info@bocascientific.com