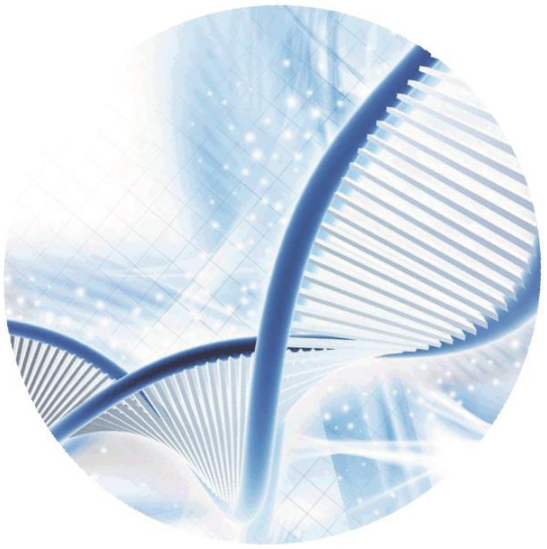


Transfection reagent

FlyFectin™

Tee Technology (Triggered Endosomal Escape)

Highly efficiency and reproducible transfection of insect cells

Protocol

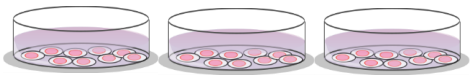
OZBIOSCIENCES
The art of delivery systems

FlyFectin™ Quick Protocol

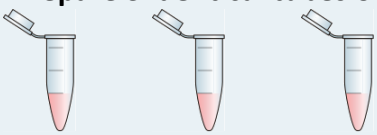
transfection/co-transfection

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

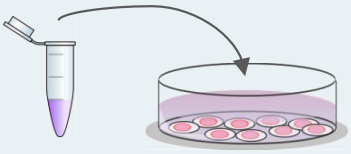
3 — **Prepare 3 tubes of FlyFectin™ (with 3 different amounts of reagent)**

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*

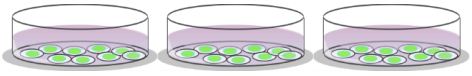
4 — **Mix each tube of DNA (step 2) to each tube of FlyFectin™ (step 3)**

5 — **Incubate 20 min at room temperature**

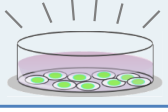
6 — **Distribute each mix dropwise onto the cells to insure uniform distribution**



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



8 — **Choose the best ratio DNA:FlyFectin™**



These conditions might require some further optimizations depending on your cells, DNA, RNA, etc.

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ 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 CO_2.
- ✓ Allow reagents to reach RT and gently vortex them before forming complexes.
- ✓ 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.
- ✓ For doses of FlyFectin less than 1µL, dilute the reagent with deionized water.
- ✓ 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.

For additional information and protocols (optimization, scaling, co-transfection…) tips, troubleshooting or other applications

www.ozbiosciences.com

Any questions?

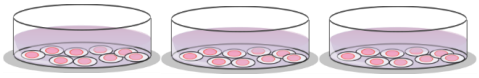
tech@ozbiosciences.com

FlyFectin™ Quick Protocol

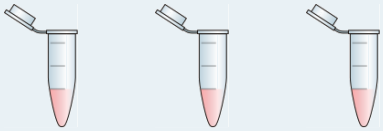
for Baculovirus

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 to mix your viral DNA and your recombinant transfer plasmid.

1 **One to 4 hours (suspension cells) or 18 to 24h (adherent cells) before transfection, seed the cells in a 60 or 100 mm petri dish (50-80% confluency day of transfection)**

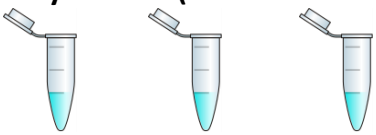


2 **Prepare 3 identical tubes of DNA containing linearized viral DNA & recombinant transfer plasmid***



60 mm	100 mm
0.84µg linearized viral DNA 4.2µg recombinant transfer plasmid in 100µL of serum-free medium or buffer* x 3	1.25µg linearized viral DNA 6.3µg recombinant transfer plasmid in 150µL of serum-free medium or buffer* x 3

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



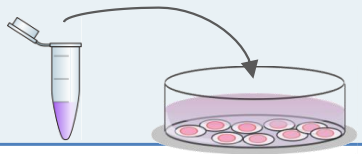
24 well plate	6 well plate
15µL/25µL/35µL in 50µL of serum-free medium or buffer*	22.5µL/37.5µL/52.5µL in 150µL of serum-free medium or buffer*

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

5 **Incubate 15 to 20 min at room temperature**

6 **During the incubation time for the complexes above, change the cells culture medium with fresh medium without antibiotics (with or without serum)***

7 **Distribute each mix dropwise onto the cells to insure uniform distribution, incubate 4 to 6h and add complete culture medium (with antibiotics)***



8 **Incubate cells for 24 to 72h at 37°C until evaluation of transgene expression and choose the best ratio DNA:FlyFectin™**

These conditions might require some further optimizations depending on your cells, DNA, RNA, etc.

* Please refer to the following section "Important Notes"

IMPORTANT NOTES – Before you begin

- ✓ 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.
- ✓ Allow reagents to reach RT and gently vortex them before forming complexes.
- ✓ **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.
- ✓ For doses of FlyFectin less than 1µL, dilute the reagent with deionized water.
- ✓ We recommend respecting the order of addition; add the DNA solution into the FlyFectin solution.
- ✓ **Step 6**: During incubation remove the medium from the cell dishes and rinse cells growing in the Petri plate(s) twice with 2 mL or 4mL of culture medium without antibiotics (be careful to not disrupt the cell monolayer and to keep the cells moist). Add very carefully 1 mL or 2mL of antibiotics-free medium to the cell monolayer (with or without serum).
- ✓ **Step 7:** Add 1 or 2 mL of complete medium (containing serum- and antibiotics) to the cells
- ✓ 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 case of cells very sensitive to transfection, the medium can be changed after 3-4 hours.

FlyFectIN Reagent |Specifications

Package content	FF50500: 500µL of FlyFectIN FF51000: 1mL of FlyFectIN FF55000: 5 x 1mL of FlyFectIN
Shipping conditions	Room Temperature
Storage conditions	Store the FlyFectIN transfection reagent at +4°C upon reception
Shelf life	1 year from the date of purchase when properly stored and handled
Product description	FlyFectIN is a high efficiency transfection reagent specifically designed to obtain highly efficient and reproducible transfection of insect cells.
Important notice	For research use only. Not for use in diagnostic procedures

Protocol | DNA

1. Cells preparation

It is recommended to plate the cells the day prior transfection in classical culture medium, or 1h before transfection in serum free medium at 4 x 10^5 cells/mL Cells should be 60-80 % confluent at the time of transfection.

2. DNA/FlyFectin complexes preparation

a. *FlyFectin*: Vortex the reagent and dilute the indicated quantity of FlyFectin (see Table 1) in 50 to 200 µL of culture medium without serum and supplement.

b. *DNA*: Dilute the indicated quantity of DNA (see Table 1) in 50 to 200 µL of culture medium without serum and supplement.

c. Add the DNA solution to the FlyFectin solutions by vigorous pipetting and incubate at room temperature for 15 to 20 minutes.

3. Transfection

a. Wash the cells twice with HBS or serum free medium

b. Add the FlyFectin / DNA complexes onto cells drop by drop and gently rock the plate to ensure a uniform distribution.

c. Complete up to Transfection volume (Table 1) with serum free medium

Tissue Culture Dish	DNA Quantity (µg)	FlyFectin Volume (µL)	Dilution Volume (µL)	Transfection Volume
24 well	1	6	2 x 50	500 µL
6 well	2	12	2 x 100	2 mL
35 mm	2	12	2 x 100	2 mL
60 mm	5	32	2 x 100	5 mL
100 mm	7	48	2 x 200	12 mL

Table 1: DNA amount, FlyFectin volume and transfection conditions suggested

d. Cultivate the cells under standard conditions until evaluation of transgene expression (usually after 48h).

NOTES: Transfection medium can be replaced by complete growth medium 4 to 8h after transfection

Protocol | for Baculovirus Expression System

1. Cells preparation

a. The day before transfection split the cells so they are in good condition on the day of transfection. Seed them at 2-3 x 10^6 cells in a 60 mm Petri dish.

b. Incubated overnight in culture medium with 10% serum.

2. DNA/FlyFectIN complexes preparation

a. *DNA preparation for Baculovirus Expression System*. Mix 100 ng of linearized viral DNA and 400 ng of recombinant transfer plasmid in serum- and antibiotics-free medium to a total volume of 30 µL solution.

b. *FlyFectin preparation*. Dilute 3 to 7 µL of FlyFectin™ in 30 µL serum- and antibiotics-free medium for each transfection.

c. Add the DNA solution to the FlyFectin™ solution immediately, mix gently and incubate at room temperature for 15 to 20 minutes.

3. Transfection

a. After the formation of FlyFectin™ /DNA complexes, add 440 µL of antibiotics-free medium to this mixture and mix very gently.

NOTES: Total volume = 500 µL (60 µL of complexes plus 440 µL of medium).

b. Add the total 500 µL to the 1mL of medium covering the cells. Washing the culture medium before adding the complexes to the cells is optional. For suspension cells, if medium changed is required, first pellet your cells (light centrifugation) and then proceed to change the culture medium.

c. Incubate 4 to 6h.

d. Add fresh complete medium (containing serum- and antibiotics) to the cells.

e. Incubate for 72h at 27 °C and harvest the virus from the cell culture medium.

IMPORTANT CONSIDERATIONS

- Cells should be 50 % confluent at the time of transfection (step 1).
- The amount of FlyFectin™ can be optimized within the range of 1.5 to 25 µL per 1 µg of nucleic acids (step 2).
- The best exposure to the transfection mixture is dependent on the sensitivity of the transfected cells. In case of very sensitive cells, they should be washed twice (remove the complexes) prior to adding the fresh medium and culturing for 72 hours (steps 3).
- Cells successfully transfected can be checked visually with an inverted microscope at 250-400 magnifications. Sometimes, the viral gene can be observed under such magnification as viral occlusions in transfected cells (crystals). In other cases, a positive sign of transfection is a 25-50 % increase of cell diameters and cell lysis.
- Virus plaque assay: The infectious potency of a baculovirus stock solution can be assayed by observing and counting plaques in an immobilized monolayer culture. Many variations of this technique are used, depending on cell line, nature of recombinant construct and identification method. Commonly used identification methods are X-Gal, X-Gluc or Neutral Red staining.

Additional Products for your transfection experiments in specific cells

- **VeroFect** for DNA transfection in Vero cells
- **COSFect** for transfection of Cos lineage cells

Purchaser Notification

Limited License
The purchase of the FlyFectIN 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 FlyFectIN 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 FlyFectIN kit reagents and documentation to OZ Biosciences, or by destroying all FlyFectIN components. Purchasers are advised to contact OZ Biosciences with the notification that a FlyFectIN 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 FlyFectIN 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
FlyFectIN 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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Rev. 06/2019