FlyFectin™

INSTRUCTION MANUAL

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FlyFectin™

Instruction Manual

FlyFectin™
The only specifically designed and very efficient transfection reagent for insect cells

List of FlyFectin™ Kits

Catalog Number	Description	Volume (µL)	Size (number of transfection / µg of DNA)
FF50500	FlyFectin ™	500	125
FF51000	FlyFectin ™	1000	250
FF55000	FlyFectin ™	5 X 1000	1250

Use the content of the table above to determine the appropriate catalog number for your needs. You can order these products by contacting us. For all other supplementary information, do not hesitate to contact our dedicated technical support: tech@ozbiosciences.com.

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1. Technology

1.1. Description

Congratulations on your purchase of the FlyFectin™ reagent!
FlyFectin™ is a unique and very efficient transfection reagent based on an aqueous formulation of cationic lipids. It has been specifically designed to achieve very high transfections of insect cells.

FlyFectin™ presents several advantages:

- Very High transfection efficiency
- Excellent reproducibility
- Fast and easy procedure
- Compatible with and without serum-containing culture media
- Multipurpose (various types of nucleic acid)
- Non toxic & economical

1.2. Kit Contents

OZ Biosciences offers three sizes of FlyFectin™ reagents. Kit contents vary according to their size.

- One tube containing 500 L of FlyFectin™ good for 100 transfections with 1 g of DNA
- One tube containing 1 mL of FlyFectin™ good for 200 transfections with 1 g of DNA
- 5 tubes containing 1 mL of FlyFectin™ good for 1000 transfections with 1 g of DNA

Stability and Storage

Storage: +4°C. Upon receipt and for long-term use, store all reagent tubes in the fridge. FlyFectin™ kits are stable for at least one year at the recommended storage temperature.

- **DO NOT FREEZE THE FlyFectin™ FORMULATION!**
- **DO NOT ADD ANYTHING TO THE STOCK SOLUTION OF FlyFectin™ FORMULATION!**

Shipping condition: Room Temperature

2. Applications

2.1. Application Areas

FlyFectin™ reagent has been especially developed to transfect insect cells. The baculovirus expression system is one of the most powerful approaches for eukaryotic recombinant protein expression. FlyFectin™ allows reaching high level of protein expression. FlyFectin™ has been developed for very efficient transfection of various types of nucleic acids such as **DNA, mRNA, siRNA or oligonucleotides** in a wide variety of insect cells. This product is intended for research purpose only.

2.2. Cell Types

FlyFectin™ has been effectively used with several insect cells.

Ag55, Anso, Asd43, Bm5, CI8, Cpp512, High5, IPBL-SF21, Kc167, Ld652, Mos20, S2, Sf9, SL-2, SL-3, SPC-SL52.

3. General Protocols

3.1. General Considerations

The instructions given below represent a sample protocol that was applied successfully with Sf9 cells and it is ideal for use of a baculovirus expression system (BES). For customer's specific applications, optimal conditions may vary depending on nucleic acid amounts, cell types and DNA / FlyFectin™ ratio. FlyFectin™ can be used both in the presence or the absence of serum. You can use your routine culture medium for the transfection, except during preparation of the FlyFectin™ / DNA complexes.

- **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.
- **Nucleic acids** should be as pure as possible. Endotoxins & traces of cesium levels must be very low since they interfere with transfection efficiencies. Moreover, we suggest avoiding long incubation time of the DNA/RNA solution in buffers or serum free medium before the addition of FlyFectin™.
- **Medium**. Do not use serum and supplement containing medium for the preparation of the FlyFectin™ / DNA complexes.

3.2. Protocol

1) For each transfection, seed 2.0 to 3.0 x 10^6 cells in a 60 mm Petri plate. After pipetting the cells into the plates, rock gently side-to-side to ensure an even monolayer. **Avoid the formation of clamp into the center of the dish**.

2) Allow the cells to attach completely to the plate or flask (3 to 4 hours at 27 °C). You can verify that cells have attached by inspecting them under an inverted microscope.

3) Complexes formation

A - For transfection:

- DNA Solution: Dilute 2 g of DNA in 100 L of culture medium without serum and antibiotics
- FlyfectIN Solution: Dilute 6 to 14 L of FlyfectIN in 100 L of culture medium without serum and antibiotics

B - For co-transfections:

- DNA Solution: Prepare the following mixture in a 1.5 mL sterile polystyrene tube:
Mix 100 ng of linearized viral DNA and 500 ng of recombinant transfer plasmid in serum- and antibiotics-free medium to a total volume of 30 L solution.
- FlyfectIN Solution: Dilute 3 to 7 L of FlyFectin™ in 30 L serum- and antibiotics-free medium for each transfection.

4) Add the DNA solution to the FlyFectin™ solution immediately, mix gently and incubate at room temperature for 15 to 20 minutes. Do not vortex!

5) During incubation remove the medium from the cell dishes and rinse cells growing in the Petri plate(s) twice with 2 mL of culture medium without antibiotics (be careful to not disrupt the cell monolayer and to keep the cells moist). Add very carefully 1 mL of antibiotics-free medium to the cell monolayer (with or without serum).

6) Add the complexes into the cells

7) Incubate 4 to 6 hours.

8) Add 1 mL of complete medium (containing serum- and antibiotics) to the cells.

9) Incubate for 72 hours at 27°C.

Important Notes:

- Cells should be 50 % confluent at the time of transfection (**step 1**).

- Optional (**step 3**):
  - ✓ *Positive control*: A plasmid encoding for -Galactosidase or -Glucuronidase (500 ng) can be used instead of the recombinant transfer plasmid as a positive control. Therefore, the plaques successfully transfected can be seen by X-Gal or X-Gluc staining.
  - ✓ *Negative control*: Replace the recombinant transfer plasmid with only buffer solution such as TE. Consequently, the background level of non-recombinants derived from uncut nucleic acid can be detected.

- The amount of FlyFectin™ can be optimized within the range of 1.5 to 25 µL per 1 µg of nucleic acids (**step 4**).
- 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 6 mL of fresh medium and culturing for 72 hours (**steps 9-10**).
- 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 (for staining solution, see our related products at the end of this instruction manual).

3.3. Troubleshooting

Our dedicated and specialized technical support group will be pleased to answer any of your requests and to help you with your transfection experiments. tech@ozbiosciences.com. In addition, do not hesitate to visit our website www.ozbiosciences.com and the FAQ section.

4. Related Products

Description
MAGNETOFECTION TECHNOLOGY
Super Magnetic Plate *(standard size for all cell culture support)* Mega Magnetic plate *(mega size to hold 4 culture dishes at one time)*
Transfection reagents:
PolyMag Neo (*for all nucleic acids*)
Magnetofectamine™ kit: Lipofectamine™ 2000 + CombiMag (*for all nucleic acids*)
NeuroMag *(dedicated for neurons)*
SilenceMag *(for siRNA application)*
Transfection enhancer:
CombiMag *(to improve any transfection reagent efficiency)*
Viral Transduction enhancers:
ViroMag (*to optimize viral transduction*)
ViroMag R/L *(specific for Retrovirus and Lentivirus)*
AdenoMag *(for Adenoviruses)*
In vivo Magnetofection
In vivo ViroMag (for magnetic assisted viral infection)
In vivo PolyMag (polymer-based magnetic nanoparticles)
In vivo DogtorMag (lipid-based magnetic nanoparticles)
LIPOFECTION TECHNOLOGY (LIPID-BASED)
Lullaby (*siRNA transfection reagent)*
DreamFect Gold *(Transfection reagent for all types of nucleic acids)*
VeroFect *(for Vero cells)*
Ecotransfect *(Economical reagent for routine transfection)*
FlyFectin *(for Insect cells)*
i-MICST TECHNOLOGY
Viro-MICST (*to transduce directly on magnetic cell purification columns)*
3D TRANSFECTION TECHNOLOGY
3DfectIN *(for hydrogels culture)*
3Dfect *(for scaffolds culture)*
RECOMBINANT PROTEIN PRODUCTION
HYPE-5 Transfection Kit (*for **H**igh **Y**ield **P**rotein **E**xpression)*
PROTEIN DELIVERY SYSTEMS
Ab-DeliverIN *(delivery reagent for antibodies)* Pro-DeliverIN *(delivery reagent for protein in vivo and in vitro)*
PLASMIDS PVECTOZ
pVectOZ-LacZ / pVectOZ-SEAP / pVectOZ-GFP / pVectOZ-Luciferase
ASSAY KITS
Bradford – Protein Assay Kit MTT cell proliferation kit -Galactosidase assay kits (CPRG/ONPG)
BIOCHEMICALS
D-Luciferin, K^+ and Na^+ 1g G-418, Sulfate 1g
X-Gal powder 1g

Please, feel free to contact us for all complementary information and remember to visit our website (www.ozbiosciences.com) to stay informed on the latest breakthrough technologies and updated on our complete product list.

Purchaser Notification

Limited License

The purchase of the ***FlyFectin***™ Reagent 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*** ™ Reagent. To inquire about such licenses, or to obtain authorization to transfer or use the enclosed material, contact the Director of Business Development at OZ Biosciences.

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Product Use Limitations

The ***FlyFectin*** ™ Reagent 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.

For more information, or for any comments on the terms and conditions of this License, please contact:

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