

FectoFly™

***In vitro* Transfection Protocol**

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Company Information

Technical Assistance

Contact the Polyplus technical support *via*:
The Polyplus website: www.polyplus-transfection.com
Email: support@polyplus-transfection.com
Phone: + 33 (0)3 90 40 61 87

Trademarks

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Related reagents

Fecturin™ for protein and virus production in mammalian cells grown in synthetic media and jetPEI™ for Biomufacturing.

POLYPLUS-TRANSFECTION SA
Bioparc
Boulevard Sébastien Brant
BP 90018
67401 ILLKIRCH cedex,
FRANCE
Phone : +33 3 90 40 61 80
Fax: + 33 3 90 40 61 81

POLYPLUS-TRANSFECTION Inc.
1251 Ave of the Americas, 34th fl.
NEW YORK, NY 10020,
USA
Phone: +1 508 315 9629

Product information

Description

FectoFly™ reagent has been developed for efficient transient transfection of insect cells and high level expression of protein or baculovirus production.

FectoFly™ has been specifically designed for Schneider cells (S2) derived from *Drosophila melanogaster*, *Spodoptera frugiperda* (Sf21, Sf9) and *Tricoplusia ni* (Tn5) cell lines; the reagent as also been shown to be highly efficient in other cell lines derived from *Bombyx mori*, *Helicoverpa zea*, *Heliothis virescens* and *Aniticarsia gemmatalis*.

Product information

Cat #	FectoFly™ reagent	150 mM NaCl buffer	Number of transfections
112-01N	0.1 ml	10 ml	10 – 20 transfections in 6-well plates
112-10N	1 ml	50 ml	100 – 200 transfections in 6-well plates
112-40N	4 x 1 ml	4 x 50 ml	400 – 800 transfections in 6-well plates

Content

1 ml of FectoFly™ transfection reagent is sufficient to perform 100 - 200 transfections in 6-well plates.

Reagent use and Limitations

For research use only. Not for use in humans.

Formulation and Storage

FectoFly™ is provided in sterile apyrogenic water. FectoFly™ and its buffer are shipped at 4°C and should be stored at 4°C. Do not freeze. FectoFly™, as guaranteed by the Certificate of Analysis, will be stable for at least one year when stored appropriately.

Quality control

Each batch of FectoFly™ is tested in a transfection assay using insect cells.

Reagents required

A 150 mM NaCl sterile solution is required to dilute both FectoFly™ reagent and DNA, the buffer is provided with the reagent.

1. Transient transfection protocol for adherent insect cells

1.1 Cell seeding

The cell density is a critical parameter for efficient insect cells transfection. In order to achieve optimal transfection efficiency with FectoFly™, prepare a **cell suspension** at **1.25 x 10⁶ cells/ml** in medium with serum. The volume to dispense per well is shown in Table 1.

Table 1. Recommended volume of cell suspension to seed 2 h before transfection

Culture vessel	Volume of cell suspension to add per well	Corresponding number of cells per well
96-well	80 µl	1 x 10 ⁵
24-well	400 µl	5 x 10 ⁵
6-well/35 mm	1.6 ml	2 x 10 ⁶
10 cm	4 ml	5 x 10 ⁶

1.2 Transfection protocol

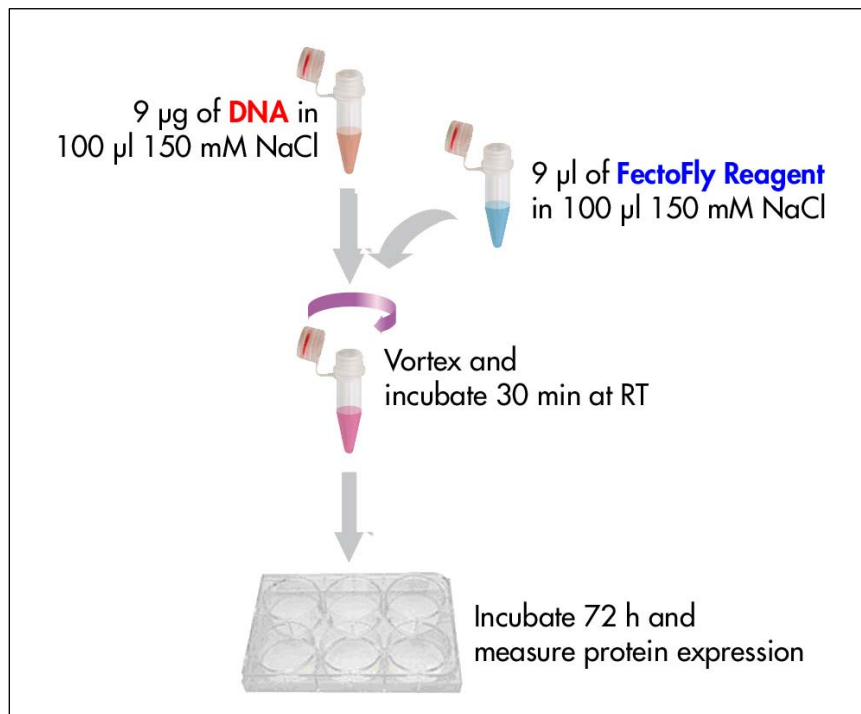
We recommend using a **1:1 ratio** DNA to FectoFly™ (w/v).

1 µg of DNA per µl of FectoFly™

Per well in 6-well plates:

1. Seed 1.6 ml of a cell suspension at 1.25 x 10⁶ cells/ml in medium containing serum.
2. Allow cells to attach for 2 h in the incubator.
3. Dilute 9 µg DNA into 100 µl 150 mM NaCl, mix by pipeting up and down or by vortexing.
4. Dilute 9 µl FectoFly™ into 100 µl 150 mM NaCl, mix.
5. Add 100 µl FectoFly™ to 100 µl DNA (in this order), vortex for 10 s and incubate for 30 min at RT.
6. Add 200 µl FectoFly™/DNA complexes dropwise to the cells to distribute the complexes evenly.
7. Gently rock the plates back and forth and from side to side.
8. Return the plates to the incubator for 4 h.
9. Add 3 ml of medium with serum (and antibiotics/antimycotics if required).
10. Proceed with protein purification after 72 h or as required.

Protocol for 6-well plates using FectoFly™



Tip: To facilitate medium removal at the time of analysis, for semi-adherent cells such as S2, centrifuge the tissue culture plate for 5 min at 180 g.

For other culture formats, apply the volumes and amounts indicated in Table 2 to the 6-well plates protocol.

Table 2. Amount of DNA and volumes of reagent to be used for transfection of most insect cells according to the cell culture formats

Culture vessel	Amount of DNA (µg)	Volume of FectoFly™ 1:1 ratio (µl)	Range of FectoFly™ for optimisation (µl)	Volume of 150 mM NaCl for DNA and FectoFly™ (µl)	Total volume of complexes added per well (µl)	Volume of medium to add after 4 h
96-well	0.75	0.75	0.5 - 1	10	20	120 µl
24-well	3	3	2.5 - 3.5	50	100	500 µl
6-well	9	9	7 - 11	100	200	2 ml
10 cm	25	25	20 - 30	500	1000	8 ml

If optimization is required in 6-well plates, test 6 to 9 µg of DNA.

1.3 Specific Transfection protocol for Tn5 cells

The protocol is comparable to the standard protocol except for the number of cells to seed, which is reduced. Since Tn5 cells are larger, for optimal transfection efficiency with FectoFly™ and Tn5 cells grown adherently, we recommend preparing a **cell suspension** at **5 x 10⁵ cells/ml** in medium containing serum. The volume to dispense per well is shown in Table 3.

Table 3. Recommended volume of Tn5 cell suspension to seed 2 h before transfection

Culture vessel	Volume of Tn5 cell suspension to add per well	Corresponding number of cells per well
96-well	80 µl	4 x 10 ⁴
24-well	400 µl	2 x 10 ⁵
6-well/35 mm	1.6 ml	8 x 10 ⁵
10 cm	4 ml	2 x 10 ⁶

Proceed as in the standard protocol described in section 1.2. using as before 1 µg of DNA and 1 µl of FectoFly™.

2. Transfection protocol for insect cells grown in suspension

2.1 Cell seeding

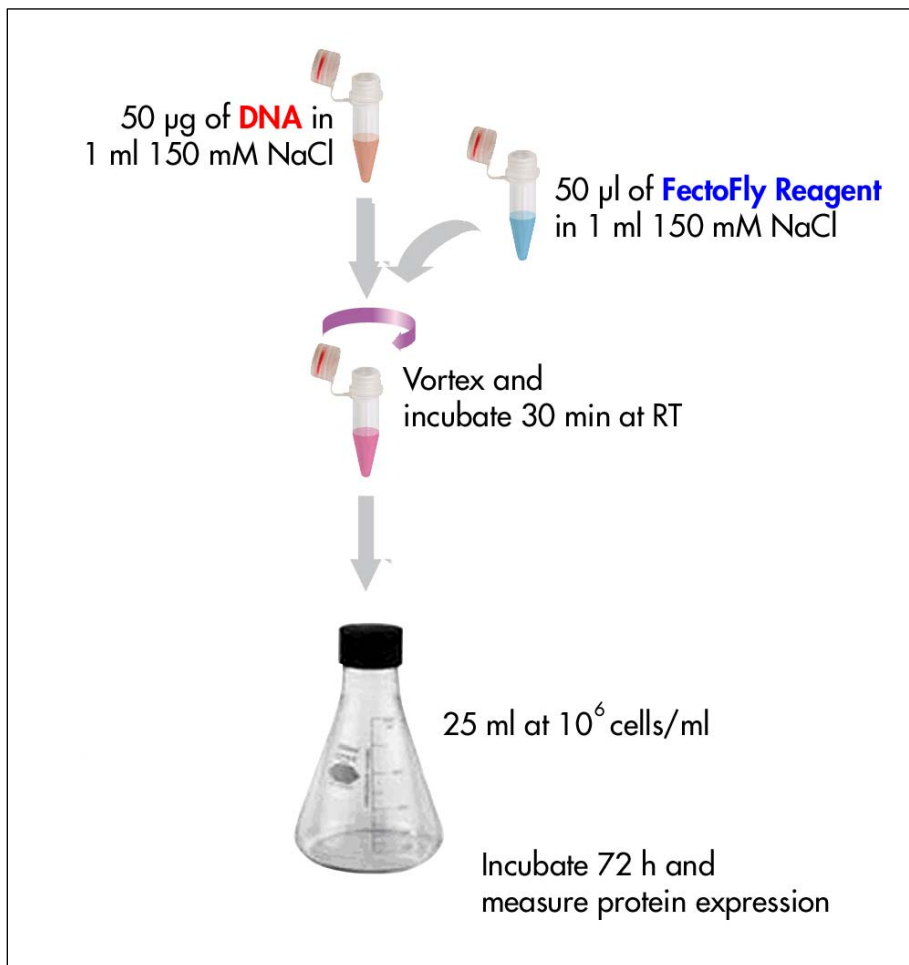
The cell density is a critical parameter for efficient insect cells transfection. In order to achieve optimal transfection efficiency with FectoFly™, we recommend preparing a cell suspension at 10⁶ cells/ml in synthetic or classical medium and antibiotics if needed. Keep under agitation at 50 to 100 rpm.

2.2 Transfection protocol

We recommend using a **1:1 ratio** DNA to FectoFly™ (w/v).

Per ml of culture, use 2 µg of DNA and 2 µl of FectoFly™

Protocol for 25 ml cell culture using FectoFly™



Per ml of cell culture volume:

1. Seed the required volume of cell suspension at 10^6 cells/ml. Keep under agitation.
2. Dilute the DNA in 150 mM NaCl as indicated in Table 3, mix by pipeting up and down or by vortexing.
3. Dilute FectoFly™ in 150 mM NaCl as indicated in Table 3, mix.
4. Add the FectoFly™ solution to the DNA solution, vortex for 10 s and incubate for 30 min at RT.
5. Add the FectoFly™/DNA complexes to the cells.
6. Incubate for 24 to 72 h under agitation in standard cell culture conditions.
7. Proceed with protein purification as required.

Table 4. Volume of complexes to prepare according to the chosen volume of culture

Volume of cell suspension	Size of cell culture vessel	Volume of 150 mM NaCl for both DNA and FectoFly™	Volume of complexes added to the cells
3 ml	50 ml tube	100 µl	200 µl
25 ml	250 ml erlenmeyer	1 ml	2 ml
50 ml	250 ml erlenmeyer	2 ml	4 ml
1 L	bioreactor	40 ml	80 ml

2.3 Specific Transfection protocol for Tn5 cells

For transfection of Tn5 cells grown in suspension, the protocol is the same as in 2.2 except for the number of cells to seed. In order to achieve optimal transfection efficiency with FectoFly™ in Tn5 cells in suspension, we recommend preparing a **cell suspension at 3×10^5 cells/ml** in synthetic or classical medium containing antibiotics. Keep under agitation at 50 to 100 rpm.

Proceed as in the standard protocol described in section 2.1.b using as before 2 µg of DNA and 2 µl of FectoFly™ per ml of culture.

Troubleshooting

Observations	Comments and Suggestions
Low transfection efficiency	<ul style="list-style-type: none"> • Optimise the amount of plasmid DNA used in the transfection assay by testing a various amounts of DNA. • Use high-quality plasmid preparation, free of RNA (the OD_{260/280} ratio should be greater than 1.8). • Check the agitation (50 -100 rpm) used for cell culture. • Optimize the FectoFly™/DNA ratio from 1:1 to 1:2. • Perform a positive control transfection experiment with a well-characterised reporter gene (Luciferase or GFP expressed from commercially available plasmid). • Test other buffers (i.e. culture medium). • Seed less cells per well. • Check culture conditions and passage cells at lower density.
Low protein yields	<ul style="list-style-type: none"> • Try a different culture medium. • Check the toxicity of the protein.