Polyplus transfection™ THE DELIVERY EXPERTS

jetPEI®

in vitro nucleic acid transfection reagent

SUPPLEMENTARY PROTOCOL

TRANSFECTION PROTOCOL FOR INSECT CELLS

Polyplus-transfection S.A. - Bioparc - Bd S. Brant - - 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 – 3rd fl. - New-York - NY 10020 - USA
www.polyplus-transfection.com

1 TRANSIENT TRANSFECTION PROTOCOL FOR ADHERENT INSECT CELLS

1.1 CELL SEEDING

Cell density is a critical parameter for efficient insect cells transfection. In order to achieve optimal transfection efficiency with jetPEI®, prepare a **cell suspension** at **1.25 x 10^6 cells/ml** in medium with serum (if used). 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
100 mm / flask 75 cm^2	4 ml	5 x 10^6

1.2 TRANSFECTION PROTOCOL

Standard conditions

1:3,3 ratio (w/v)

for 1 µg of nucleic acid use 3.3 µl of jetPEI®

The following conditions are given per well of a 6-well plate.

1. Seed 1.6 ml of a cell suspension at 1.25 x 10^6 cells/ml in medium containing serum if used.
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 30 µl jetPEI® into 100 µl 150 mM NaCl, mix.
5. Add 100 µl jetPEI® to 100 µl DNA (in this order), vortex for 10 s and incubate for 15-30 min at RT.
6. Add 200 µl jetPEI®/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.
9. Four hours later, add 2 ml of growth medium
10. Proceed with protein analysis after 72 h or as required.

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 adherent insect cells according to the cell culture formats

Culture vessel	Amount of DNA (µg)	Volume of jetPEI® 1:3.3 ratio (µl)	Range of jetPEI® for optimisation (µl)	Volume of 150 mM NaCl for DNA and jetPEI® (µl)	Total volume of complexes added per well (µl)	Volume of medium to add after 4 h
96-well	0.75	2.5	1.65-3.3	10	20	120 µl
24-well	3	10	8.25 – 11.55	50	100	500 µl
6-well	9	30	23 – 33.3	100	200	2 ml
10 cm	25	82.5	66-100	500	1000	8 ml

If optimization is required, test 6 to 9 µg of DNA per well of a 6-well plate.

1.3 SPECIFIC TRANSFECTION PROTOCOL FOR TN5 CELLS

The protocol is comparable to the standard protocol except for the number of cells to seed. Since Tn5 cells are larger, for optimal transfection efficiency with jetPEI® and Tn5 cells grown adherently, we recommend preparing a **cell suspension** at **5 x 10^5 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.

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 jetPEI®, we recommend preparing a cell suspension at **10^6 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 **1:3.3 DNA to jetPEI® ratio** (w/v).

Per ml of culture, use 2 µg of DNA and 6.6 µl of jetPEI®

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 jetPEI® in 150 mM NaCl as indicated in Table 3, mix.
4. Add the jetPEI® solution to the DNA solution, vortex for 10 s and incubate for 15-30 min at RT.
5. Add the jetPEI®/DNA complexes to the cells.
6. Incubate for 24 to 72 h under agitation in standard cell culture conditions.
7. Proceed with protein analysis as required.

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

Volume of cell suspension	Volume of 150 mM NaCl for both DNA and jetPEI®	Volume of complexes added to the cells
3 ml	100 µl	200 µl
25 ml	1 ml	2 ml
50 ml	2 ml	4 ml
1 L	40 ml	80 ml

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 jetPEI® in Tn5 cells in suspension, we recommend preparing a **cell suspension** at **3 x 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 2.2. using as before 2 µg of DNA and 6.6 µl of jetPEI® per ml of culture**.**