Transfection reagent

Cat. No. 300101 **Volume 0.15 ml**
Cat. No. 300102 **Volume 1.0 ml**

Storage: +4°C. Stable for 1 year. Shipped at room temperature.

General description
*baculo*FECTIN consists of a positively charged polymer embedded into a porous nanoparticle. *baculo*FECTIN, when bound to baculovirus DNA forms a stable nanoparticle-DNA complex and protects the DNA from nucleases. With its unique characteristics only the nanoparticle-DNA complex is preferentially taken up by the insect cells and the complex is non-toxic to insect cells.

Making recombinant baculovirus by DNA co-transfection in 35 mm^2 dishes
The following procedure is for the transfection of insect cells in 35 mm^2 dishes. Allow enough dishes for each co-transfection and a mock-transfection control dish and work under sterile conditions.

- One hour prior to the transfection seed 1 x 10^6 Sf9 cells in 2 ml of growth medium (0.5 x 10^6 cells/ml) or 1.5 × 10^6 Sf21 cells in 2 ml of growth medium (0.75 x 10^6 cells/ml) into each 35 mm^2 cell culture dish required (1 dish per virus) plus any controls
  Note: only use cells from a culture that is >95% viable and in log growth phase

- Pipette 1 ml serum-free, antibiotic-free medium into a sterile eppendorf tube (preferably a disposable polystyrene bijoux) for each co-transfection
  Note: in our experience whilst any serum-free medium may work for transfections, we recommend using a less complex medium at this stage. e.g. TC-100

- To each 1 ml add 5 µl *baculo*FECTIN (including a mock-transfection control) and mix gently

- Then to each 1 ml add 100 ng of baculovirus DNA (i.e. 5 µl *flash*BAC™ at 20 ng/µl) and either 500 ng transfer vector DNA containing the gene of interest or 500 ng of control transfer plasmid DNA (as supplied in the *flash*BAC kits: 5 µl at 100 ng/µl) and mix gently to avoid shearing the DNA. In the mock-transfection control, omit the DNA from the medium. Incubate at room temperature for 15-30 minutes to allow the nanoparticle-DNA complexes to form

- Remove the culture medium from the 35 mm^2 dishes of cells using a sterile pipette, ensuring that the cell monolayer is not disrupted. If the cells are in serum supplemented media, then wash twice with 1 ml of serum-free media

- Add the *baculo*FECTIN/ DNA mixture drop-wise into the centre of a dish of cells

- Incubate the dishes in a sandwich box overnight or for at least five hours at 28°C

- After this time, add 1 ml of your preferred insect cell culture medium to each dish (there is no need to remove transfection reagent) and further incubate at 28°C for 5 days to obtain recombinant virus

- Harvest the 2 ml culture medium (containing recombinant virus) after 5 days into a sterile tube and store in the dark at 4°C. This is your seed stock of recombinant virus

- If the pAcRP23.lacZ positive control transfer vector supplied with the *flash*BAC kit has been used to make recombinant virus the infected cells can be stained using X-gal. Add 1 ml of appropriate insect cell culture medium (or phosphate buffered saline, PBS) containing 15 µl X-gal (2% w/v in N, N Dimethylformamide; DMF) and incubate at 28°C. After ~5 hours the cells and culture medium will appear blue in colour, confirming the production of recombinant virus expressing lacZ

Making recombinant baculovirus by DNA co-transfection in 24-well plates

- Confirm the health and viability of the insect cells to be used with an inverted microscope and prepare a stock of Sf9/ Sf21 cells in serum-free, antibiotic-free medium at a cell density of 5×10^5 cells/ml

- If using a liquid handler then design a simple program for the robot to carry out:
  - cell seeding
  - co-transfection mixture preparation
  - aliquoting the co-transfection mixture into the 24 wells containing the seeded cells

- Either manually or using a robot, aliquot 400 µl (2×10^5 cells/well) into each well of a 24-well tissue culture plate. Allow the cells to settle and attach for 1 hour at room temperature

- During the 1 hour incubation period, either manually or using a robot, prepare 24 co-transfection mixes i.e. in the wells of a 96-well plate (made from polystyrene and with U- or V-shaped well)

- Dispense the following to each of 24 wells of a 96 well plate (or other suitable tubes) in the following order to give a final volume of 20 µl:
  8 µl serum-free, antibiotic-free medium
  2 µl *baculo*FECTIN
  5 µl *flash*BAC DNA (100 ng)
  5 µl transfer plasmid DNA (500 ng) containing the gene of interest (include one reaction containing the pAcRP23.lacZ positive control transfer plasmid)

- Mix the reagents by gentle pipetting (dispense and aspirate three times at slow speed). Incubate at room temperature for 15-30 minutes

- Dispense the 20 µl co-transfection mix into the appropriate wells of the 24-well plate containing the cell monolayers

- Replace the lid and seal with parafilm to prevent evaporation. Incubate at 28°C for 5 days

- After 5 days check the cells have grown and appear healthy in the non-transfected control well using an inverted microscope. Harvest the culture medium containing recombinant virus from each well and store at 4°C in the dark. This is your seed stock of recombinant virus

- Add 100 µl media containing 5 µl X-gal (2% w/v in N, N Dimethylformamide; DMF) to the cells co-transfected with the pAcRP23.lacZ positive control transfer plasmid. *Lac*Z expression should become apparent by the blue coloration produced by the β-galactosidase protein, confirming the presence of recombinant viruses

For additional information on this protocol please refer:

Possee RD , R.H., KS Richards, SG Mann, E Siaterli, CP Nixon, H Irving, R Assenberg, D Alderton, R J Owens, L A King Generation of baculovirus vectors for the high throughput production of proteins in insect cells. *Biotechnology and Bioengineering*, **2008**. 101(6):p. 1115-22

General transfection

In addition to transfecting insect cells with baculovirus DNA, *baculo*FECTIN is also effective as a general transfection reagent with any DNA and most cell types. Contact Oxford Expression Technologies or your distributor to obtain a protocol.