

# *baculo***FECTIN**<sup>TM</sup> Transfection reagent

Cat. No. 300101  
Cat. No. 300102

Volume 0.15 ml  
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<sup>2</sup> dishes

The following procedure is for the transfection of insect cells in 35 mm<sup>2</sup> 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  $1 \times 10^6$  Sf9 cells in 2 ml of growth medium ( $0.5 \times 10^6$  cells/ml) or  $1.5 \times 10^6$  Sf21 cells in 2 ml of growth medium ( $0.75 \times 10^6$  cells/ml) into each 35 mm<sup>2</sup> 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  $\mu$ l *baculo*FECTIN (including a mock-transfection control) and mix gently
-  Then to each 1 ml add 100 ng of baculovirus DNA (i.e. 5  $\mu$ l *flashBAC*<sup>TM</sup> at 20 ng/ $\mu$ l) and either 500 ng transfer vector DNA containing the gene of interest or 500 ng of control transfer plasmid DNA (as supplied in the *flashBAC* kits: 5  $\mu$ l at 100 ng/ $\mu$ l) 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<sup>2</sup> 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 *flashBAC* 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  $\mu$ l 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  $5 \times 10^5$  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  $\mu$ l ( $2 \times 10^5$  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  $\mu$ l:
  - 8  $\mu$ l serum-free, antibiotic-free medium
  - 2  $\mu$ l *baculo*FECTIN
  - 5  $\mu$ l *flash*BAC DNA (100 ng)
  - 5  $\mu$ l 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  $\mu$ l 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  $\mu$ l media containing 5  $\mu$ l X-gal (2% w/v in N, N Dimethylformamide; DMF) to the cells co-transfected with the pAcRP23.*lacZ* positive control transfer plasmid. *LacZ* expression should become apparent by the blue coloration produced by the  $\beta$ -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.