USER GUIDE to *baculoFECTIN II*

Catalogue Number	300105 – 150ul
	300106 – 1ml
Storage	Tightly capped at -20°C
Product Guarantee	1 year from the date of purchase, when properly stored and handled.

You will require:

- baculoFECTIN II 1.2µl per reaction
- 35mm sterile cell culture dishes or multi-well dishes as appropriate
- Sterile 1.5ml tubes
- Serum-free, antibiotic-free medium (e.g baculoGROW II, TC100) for complex formation and transfection stage
- *flash*BAC™ DNA and positive control transfer plasmid from a GenWay Kit (or equivalent)
- Transfer vector with gene to be expressed
- Incubator at 28°C and a clean sandwich box

Procedure:

1. One hour prior to the transfection, seed 1 x 10^6 Sf9 cells in 2ml of growth medium (0.5 x 10^6 cells/ml) or 1.5 x 10^6 Sf21 cells in 2ml of growth medium (0.75 x 10^6 cells/ml) into each 35mm cell culture dish required - 1 dish per recombinant virus plus one mock-transfection control and/or a positive-control transfection using the *lacZ* transfer plasmid in the *flash*BAC™ kit.

   Note: Check cells have settled and formed a sub-confluent monolayer before proceeding. While the cells are settling, prepare the transfection mixes [steps 2-3].

2. For each transfection, pipette 0.1ml serum-free, antibiotic-free medium into a sterile tube (preferably a disposable 1.5ml tube). Add 100ng of baculovirus DNA (e.g. 5µl *flash*BAC™ DNA at 20ng/µl) and either 500ng transfer vector DNA containing the gene of interest or 500ng of control transfer plasmid DNA (as supplied in the flashBAC™ kits: 5µl at 100ng/µl) and mix gently to avoid shearing the DNA. In the mock-transfection control, omit the DNA from the medium.

3. The baculoFECTIN II reagent should be gently vortexed for 5 seconds before adding 1.2µl to each tube containing the transfection mixture [from 2]. Mix gently and incubate at room temperature for 15-20 minutes to allow the nanoparticle-DNA complexes to form.

   Note: During this incubation stage the solution may appear cloudy due to the baculoFECTIN II interaction with the media. This does not affect the transfection efficiency of baculoFECTIN II.

4. Remove 1ml of culture medium from the 35mm dishes of cells using a sterile pipette, ensuring that the cell monolayer is not disturbed (leaving 1ml in the dish).

5. Add the 0.1ml baculoFECTIN II/DNA transfection mixture drop-wise into the centre of a dish of cells; repeat for additional viruses and the control samples as needed.

6. Incubate the dishes in a sandwich box overnight at 28°C.

7. After this time, add 1ml of your preferred insect cell culture growth medium to each dish (there is no need to remove the transfection reagent) so that each dish has 2ml medium and continue to incubate at 28°C. During this time recombinant virus particles will form and be released into the culture medium by budding.

   Note: The 1ml of insect cell culture growth medium added at this stage could be supplemented with antibiotic if preferred, e.g. 200 units/ml of penicillin and 200µg/ml of streptomycin. At this stage a fine precipitate may form on the cells. This is normal and does not affect the cells or transfection efficiency.

8. At 5 days post-transfection, harvest the 2ml culture medium (containing recombinant virus) into a sterile tube and store in the dark at 4°C (e.g. wrap in foil).

   Note: This is your seed stock (P0) of recombinant virus, which can be used to amplify a P1 virus stock by infecting a 50ml Sf9 culture (2 x 10^6 cell/ml) with 0.5ml of the seed stock. The P1 virus stock should be harvested 4-5 days post infection and the titre of the virus determined (e.g. GenWay's baculoQUANT all-in-one or by plaque-assay).

9. 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 1ml of appropriate insect cell culture medium (or phosphate buffered saline, PBS) containing 15µl X-gal (2% w/v in N, N Dimethlyformamide; 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*.

Additional documents can be found at www.genwaybio.com