*baculo*FECTIN II

An introductory guide to using *baculo*FECTIN II. Full protocols can be downloaded from our *baculo*COMPLETE User Guide at www.oetltd.com/shop.

Product Information

Product	Catalogue Number	Size
*baculo*FECTIN II	300105	150µL
*baculo*FECTIN II	300106	1mL

Kit Contents and Composition

Item	Composition	Storage
*baculo*FECTIN II	*baculo*FECTIN II transfection reagent	Tightly capped at -20°C

Product guarantee: 1 year from the date of purchase, when properly stored and handled.

Overview

*baculo*FECTIN II is a non-toxic insect transfection reagent optimised for use in insect cells to enable successful transfection and produce high titres of recombinant viruses for subsequent amplification. Although optimised for insect cell lines such as *Sf9*, *Sf21*, *T.ni* and Drosophila S2, the reagent will also work with other cell lines, including primary cells and various mammalian cell lines. *baculo*FECTIN II consists of a positively charged polymer with DNA-binding capacity embedded into a novel porous nanoparticle. The complex's unique size can be preferentially taken up by the cells whilst remaining protected by DNA degradation. The main benefits of *baculo*FECTIN II compared with conventional lipid-based products are that it has a quick, effective and easy protocol, from co-transfection to high titre seed stock. It is non-toxic so cell health and viability is maintained and the nanoparticle/polymer duplex optimizes entry into cells and stabilizes the DNA within transfected insect cells. Stable for up to a year when stored correctly, *baculo*FECTIN II is the perfect companion to our leading baculovirus expression system, *flash*BAC™.

Experimental Procedure

Updated November 2022

Required by User:

- 35mm tissue culture dishes/6-well plate seeded with a sub-confluent monolayer of *Sf*21 ($1.4x10^6$ cells/2mL) or *Sf9* cells ($1x10^6$ cells/2mL) – one dish/well for each co-transfection. You can also use a 12-well plate seeded with $0.4x10^6$ cells/mL of *Sf*21/*Sf*9 cells.
- Serum-free insect cell culture or transfection media. We recommend using TC100 as a transfection medium or use Transfection Medium [Expression Systems LLC] or Grace's Insect Medium [Gibco®].
- Insect cell culture growth media (e.g. serum-free ESF 921™ [Expression Systems LLC], Sf-900 ™ II [Gibco®] or TC100 with 10% serum)
- Sterile transfer plasmid DNA containing gene to be expressed (500ng per co-transfection)
- *flash*BAC™ baculovirus expression kit with control transfer plasmid or equivalent expression system

Method:

1. Seed the dishes/wells with cells at least 1 hour before use to allow cells to attach and recover. Cells should be taken from a log phase culture that were at least 90% viable. Observe cells under a phase contrast/bright field microscope to ensure cells are evenly distributed over the surface of the dish/well. It is recommended you set up an extra dish of cells for a null reaction, which will be absent of co-transfection mix and a mock reaction, which will be absent of DNA.

2. During the 1 hour incubation period, prepare the co-transfection mix of DNA. For each co-transfection you need to gently mix in a polystyrene tube (do not use polypropylene), in the following order:
   - 100µL transfection medium or serum-free medium (e.g. TC100 or Grace's Insect Media); **do not** use ESF 921™ or similar media.
   - 100ng virus DNA (e.g *flash*BAC™ [5µL])
   - 500ng of your own transfer vector or control plasmid (*lacZ* positive control from *flash*BAC™ kit [5µL])

   Total mix volume = 110µL.

3. The *baculo*FECTIN II reagent should be gently vortexed for 5 seconds before adding 1.2µl to each tube containing the DNA co-transfection mixture (step 2). Mix gently and incubate at room temperature for 15-20 minutes to allow the nanoparticle-DNA complexes to form. During the incubation stage the solution may appear cloudy due to the *baculo*FECTIN II interacting with the media. This does not affect the transfection efficiency of *baculo*FECTIN II.

   Total mix volume = 111.2µl.

4. If cells were maintained in serum-supplemented growth media (e.g. TC100 with serum) skip to step 6. If the cells were maintained in serum-free growth medium such as ESF 921™, simply remove and discard 1mL of medium from the 35mm dishes/6-well plate. **Do not** remove media if using a 12-well plate. All dishes/wells should at this stage contain 1mL of growth medium without any serum. Pipette the 111.2µL transfection mix from step 2 drop-wise into each dish/well, taking care to distribute the mixture across the dish/well. Incubate overnight (16-24 hours) at 28°C.

5. After overnight incubation, add an extra 1mL of serum-free growth medium to the 35mm dishes/6-well plate **or replace** the 1mL of medium in the 12-well plates with 1mL serum-free growth medium. Continue the incubation for 4 more days (5 days in total). We recommend incubating the co-transfections using the same medium the cells were initially grown in. This will prevent additional stress to the cells and help ensure maximum recombinant virus production.

6. This step **is only** for cells grown in serum-supplemented growth medium. Wash the monolayer twice with serum-free or transfection medium and then add 1mL of serum-free or transfection medium to each 35mm dish/6-well plate/12-well plate. Pipette the 111.2µL transfection mix from step 2 drop-wise into each dish/well, taking care to distribute the mixture across the dish/well. Incubate overnight (16-24 hours) at 28°C. After overnight incubation, remove all media from the 35mm dishes/6-well plate/12-well plate and replace with serum-supplemented growth medium. Continue the incubation for 4 more days (5 days in total). We recommend incubating the co-transfections using the same medium the cells were initially grown in. This will prevent additional stress to the cells and help ensure maximum recombinant virus production.

7. Harvest the culture medium containing recombinant budded virus into a sterile container and store in the dark at 4°C; this is your P0 virus stock. If the *lac*Z positive control has been used, after harvesting budded virus add 1mL of serum-free growth medium or phosphate buffered saline (PBS) containing 15µL 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 *lac*Z.

Protocols and advice on topics including virus amplification, virus titration, optimising expression, and protein purification and scale-up can be downloaded from our baculoCOMPLETE User Guide at www.oetltd.com/shop or via our blog oetltd.wordpress.com.

Product Use

Products are for research purposes only. Not for diagnostic or therapeutic use. For applications including the production of proteins for commercial or diagnostic use including clinical/therapeutic use please contact info@oetltd.com.