The BacPAK6 and BacPAK6 *Sec+* System

An introductory guide to using the BackPAK6 and BacPAK6 *Sec+* system. Full protocols can be downloaded from our *baculo*COMPLETE User Guide at www.oetltd.com/shop.

Product Information

Product	Catalogue Number	Size
BacPAK6 Linearised DNA	1001101	5 reactions
BacPAK6 Linearised DNA	1001102	24 reactions
BacPAK6 Linearised DNA	1001103	96 reactions
BacPAK6 *Sec+* Linearised DNA	1001104	5 reactions
BacPAK6 *Sec+* Linearised DNA	1001105	24 reactions
BacPAK6 *Sec+* Linearised DNA	1001106	96 reactions

Kit Contents and Composition

Item	Composition	Storage
BacPAK6/BacPAK6 *Sec+* Linearised DNA	BacPAK6/BackPAK6 *Sec+* DNA 20ng/µL suspended in Tris-EDTA buffer pH 8.0	Tightly capped at 4°C. Do not freeze
pAcOET vector	Empty transfer vector 100ng/µL suspended in Tris-EDTA buffer pH 8.0	Tightly capped at -20°C

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

Overview

BacPAK6 linear baculovirus expression kit is the original, convenient, highly efficient reagent for generating recombinant viruses. It comprises a modified Autographa californica nucleopolyhedrovirus (AcMNPV) genome which has increased recombinant virus production by 90% when compared to standard baculovirus expression vectors. The insertion of a *lacZ* gene in the place of the native polyhedrin allows for easy blue/white selection from the parental virus, while an additional chitinase (*chiA*) deletion from BacPAK6 *Sec+* significantly improves the yield and purification of secreted and membrane targeted proteins. Usually, a single plaque assay titration incubated with X-gal and counterstained with neutral red is sufficient to differentiate parental blue plaques from recombinant white/colourless plaques. These can be readily isolated and amplified to working stocks of purified recombinant virus in a few days.

Experimental Procedure

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Required by User:

- 35mm tissue culture dish/6-well plate seeded with a sub-confluent monolayer of *Sf*21 ($1.4x10^6$ cells/2mL or *Sf*9 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 S*f*21/S*f*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 containing gene to be expressed under a suitable mammalian promoter (500ng per co-transfection)
- Transfection reagent (e.g. *baculo*FECTIN II [OET], Lipofectinamine™ [Invitrogen™], FuGENE [Promega] or GeneJuice® [Novagen®])

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 and transfection reagent. For each co-transfection you need to 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 from the kit (BacPAK6/BacPAK6 *Sec+* [5µL])
- 500ng of your own transfer vector or control plasmid (pAcOET positive control from BacPAK6/BacPAK6 *Sec+* kit [5µL])
- *baculo*FECTIN II transfection reagent 1.2µL per reaction (or other suitable transfection reagent using the volume as indicated by the manufacturer)

Mix (total volume = 111.2µL) and leave at room temperature for 15 minutes.

3. 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.

4. 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.

5. 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.

6. Harvest the culture medium containing budded recombinant virus into a sterile container and store in the dark at 4°C. The next step is to isolate your recombinant virus using plaque purification. Parental BacPAK6/BacPAK6 *Sec+* virus forms blue plaques in the presence of X-gal while the recombinant virus forms white/colourless plaques.

Protocols and advice on topics including virus purification using plaque assay, virus titration and amplification, 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.