The *flash*BAC™ System

An introductory guide to using the *flash*BAC™ system including *flash*BAC™, GOLD, ULTRA and PRIME. Full protocols can be downloaded from our *baculo*COMPLETE User Guide at www.oetltd.com/shop.

Product Information

Product	Catalogue Number	Size
*flash*BAC™ 5 reactions	100150	500ng in 25µL
*flash*BAC™ 24 reactions	100151	2.4µg in 120µL
*flash*BAC™ 96 reactions	100152	9.6µg in 450µL
*flash*BAC™ Bulk	100153	-
*flash*BAC GOLD 3 reactions	100200	300ng in 15µL
*flash*BAC GOLD 5 reactions	100201	500ng in 25µL
*flash*BAC GOLD 24 reactions	100202	2.4µg in 120µL
*flash*BAC GOLD 96 reactions	100203	9.6ug in 450µL
*flash*BAC GOLD Bulk	100204	-
*flash*BAC ULTRA 3 reactions	100304	300ng in 15µL
*flash*BAC ULTRA 5 reactions	100300	500ng in 25µL

Product	Catalogue Number	Size
*flash*BAC ULTRA 24 reactions	100301	2.4µg in 120µL
*flash*BAC ULTRA 96 reactions	100302	9.6µg in 450µL
*flash*BAC ULTRA Bulk	100303	-
*flash*BAC PRIME 5 reactions	100500	500ng in 25µL
*flash*BAC PRIME 24 reactions	100501	2.4µg in 120µL
*flash*BAC PRIME Bulk	100502	-
*flash*BAC Selection Box 1 3 x 3 reactions	100400	3 x 300ng in 15µL
*flash*BAC Selection Box 2 4 x 3 reactions	100401	4 x 300ng in 15µL

Kit Contents and Composition

Item	Composition	Storage
*flash*BAC™ DNA	*flash*BAC™ DNA 20ng/µL suspended in Tris-EDTA buffer pH 8.0	Tightly capped at 4°C. Do not freeze
Control transfer vector	*lacZ* positive control DNA 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

The *flash*BAC™ system is the most advanced platform technology for the production and isolation of recombinant baculoviruses. *flash*BAC™ utilises DNA from the Autographa californica nucleopolyhedrovirus (AcMNPV) that has been genetically optimised to function as a recombinant protein expression vector. A partially deleted copy of the essential gene ORF1629 prevents the virus replicating in insect cells, whilst a bacterial artificial chromosome (BAC) at the *polh* locus allows the virus genome to be maintained in bacterial cells as a bacmid. The sequence encoding the desired gene is cloned into a suitable transfer vector under control of the powerful *polh* promoter. Homologous recombination between ORF603 and ORF1629 in *flash*BAC™ and the transfer vector removes the BAC replicon, replacing it with the target gene sequence. In the process ORF1629 is restored producing an infectious virus that is able to replicate within insect cells and generate budded virus containing the target gene.

Importantly, *flash*BAC™ has been designed to remove the need for separation of recombinant virus from parental virus, so no plaque-purification steps are needed. Further modifications to the *flash*BAC™ viral backbone have allowed for the production of more complex and 'difficult to express' proteins from insect cells.

Product	Gene Deletions	Optimised For
*flash*BAC™	Chitinase (*chiA*)	Membrane and secreted proteins
*flash*BAC GOLD	Chitinase (*chiA*), Cathepsin (*v-cath*)	Proteins susceptible to protease breakdown
*flash*BAC ULTRA	Chitinase (*chiA*), Cathepsin (*v-cath*), *p74*, *p26*, *p10*	Membrane, secreted, and complex/highly processed proteins
*flash*BAC PRIME	None	Simple nuclear, cytoplasmic proteins and VLPs targeted to the nucleus where cell lysis aids purification

Advantages of using *flash*BAC™:

- Simple and rapid one-step process amenable to high throughput systems
- Back compatible with a large range of commercial transfer vectors
- Capable of expressing a wide variety of proteins
- Cost-effective; high yields of quality protein produced in insect cells
- Genetic integrity of recombinant virus is maintained long-term

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 *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 DNA containing gene to be expressed (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 (*flash*BAC™ [5µL])
   - 500ng of your own transfer vector or control plasmid (*lac*Z positive control from *flash*BAC™ kit [5µL])
   - *baculo*FECTIN II transfection reagent 1.2µL per reaction (or other suitable transfection reagent using the volume as indicated by the manufacturer)

   Total mix volume = 111.2µL. 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 5. 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 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.

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