*flash*BAC™ Baculovirus Expression Systems

Quick Reference Protocol

Instructions for MIR 6115, 6120, 6135, 6140

Full protocol, SDS and Certificate of Analysis available at mirusbio.com/6115

SPECIFICATIONS

Storage	Store *flash*BAC™ DNA at 4°C and control plasmid at 4°C or -20°C.
Product Guarantee	1 year from the date of purchase, when properly stored and handled.

TRANSFECTION OF *flash*BAC™ + TRANSFER DNA

Full protocol and additional documentation available at ***mirusbio.com/6115***

Fill in volumes below based on culture vessel used for transfection (Table 1).

A. Plate cells approximately 1-2 hours prior to transfection

1. Plate cells in ___ml complete growth medium (per well).
   For Sf9 cells: Plate cells at a density of 0.5×10^6 cells/ml.
   For Sf21 cells: Plate cells at a density of 0.75×10^6 cells/ml.
2. Incubate cell cultures at room temperature for 1 hour.

B. Prepare *Trans*IT®-Insect Reagent: *flash*BAC™ DNA: transfer DNA complexes

1. Warm *Trans*IT®-Insect Reagent to room temperature and vortex gently.
2. Place ___µl of Grace's Insect Basal Medium (without serum) in a sterile tube.
3. Add ___µl transfer vector DNA to tube.
4. Add ___µl *flash*BAC™ DNA to tube. Mix gently by pipetting.
5. Add ___µl of *Trans*IT®-Insect Reagent. Mix gently by pipetting.
6. Incubate at room temperature for 15-20 minutes.

C. Distribute complexes to cells

1. Remove half of the culture medium from the well (leaving half volume in the well).
2. Add *Trans*IT®-Insect Reagent: *flash*BAC™ DNA: transfer DNA complex mixture drop-wise to different areas of the well.
3. Gently rock plate for even distribution of complexes.
4. Incubate cell cultures overnight at 28°C. NOTE: To prevent evaporation, wrap edges of cell culture vessel with parafilm or store in a sterile plastic box. A tray of sterile water may be placed inside the incubator to increase humidity.

D. Incubate and harvest the P0 seed stock

1. The following day, add a volume of complete insect culture medium equivalent to that removed in step C.1 to each well (e.g. add 1 ml to wells if 1 ml was removed in C.1).
2. Continue to incubate cultures at 28°C for 4 more days, observing cells for signs of infection such as enlarged nuclei and a lack of confluency (as compared to controls).
3. At 5 days post-transfection, centrifuge cell culture(s) at 300 × g for 5 minutes to pellet the cells.
4. Transfer the supernatant containing recombinant baculovirus to a new sterile tube. Store at 4°C, protected from light. This is your P0 seed stock.

Table 1. Recommended starting conditions

Culture vessel	24-well plate	12-well plate	6-well plate
Surface area	1.9 cm^2	3.8 cm^2	9.6 cm^2
Complete growth medium	0.4 ml	0.8 ml	2 ml
Serum-free medium	20 µl	40 µl	100 µl
***flash*BAC™ DNA (20 ng/µl stock)**	1 µl	2 µl	5 µl
Transfer DNA (500 ng/µl stock)	0.2 µl	0.4 µl	1 µl
***Trans*IT®-Insect Reagent**	0.24 µl	0.48 µl	1.2 µl

Verifying Infection Efficiency

If the pAcRP23.lacZ positive control transfer vector was used to make recombinant virus, the infected cells can be stained to verify expression using X-gal. A titer of approximately 1×10^7 pfu/ml at day 5 is expected when following the steps in this protocol.

For additional information, see the full protocol at ***www.mirusbio.com/6115***

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AMPLIFICATION OF RECOMBINANT VIRUS

Full protocol and additional documentation available at **mirusbio.com/6115**

The recombinant P0 virus seed stock produced in the previous section must be further amplified prior to protein expression experiments. The following workflow will amplify 50-100 ml virus using the seed stock as inoculum. Use proper aseptic technique for the following procedures.

A. Maintain Sf9 or Sf21 cell cultures at an appropriate density

1. Maintain insect cells to ensure that they are in log growth phase prior to baculovirus amplification.
2. Seed 50-100 ml of Sf9 or Sf21 cells at an appropriate density for amplification. The cell density will vary with each cell type and method of culture. For example:

   Cells in serum-free medium: Seed in shake flasks at 2×10^6 cells/ml.

   Cells in serum-supplemented medium: Seed in spinner flasks at 0.5×10^6 cells/ml.

   NOTE: Do not exceed 50% of the recommended volume for the flask. For example, if using a 250-ml Erlenmeyer shake flask, do not use a culture volume greater than 125 ml.

B. Inoculate insect cell cultures with P0 baculovirus seed stock

1. Add 0.5 ml of the recombinant virus seed stock (P0) to the flask containing 50-100 ml of cells at A density of 2×10^6 cells/ml (if in serum-free conditions).
2. Incubate the cells at 28°C, shaking at 130-140 rpm, for 3-4 days.

NOTE: Baculovirus-infected cells will appear grainy, rounded and enlarged with enlarged nuclei.

3. When the cells appear infected with virus, harvest the culture medium by centrifugation at 3,000 rpm, at 4°C for 15 minutes.
4. In a laminar flow hood, pour the supernatant into a sterile container. Store the recombinant virus at 4°C, protected from light.

NOTE: Baculovirus stocks may be stored for up to 12 months at 4°C, though loss of titer can happen earlier. To minimize titer loss, add 2-5% serum. If virus has been stored for greater than 3 months, titer the virus before use and re-amplify if necessary. For long-term storage, make aliquots of virus stocks and store at -80°C. Due to reduction of viral titer by freezing, multiple freeze thaws should be avoided and virus should be re-amplified before use. Storage at -20°C or in liquid nitrogen is not recommended.

Determining Virus Titer

To minimize variability and ensure accurate multiplicity of infection (MOI), it is important that the titer of the virus be determined. A titer of 5×10^7 pfu/ml or higher is generally adequate for gene expression. Please see Section III of the full protocol for Plaque Assay instructions which can be used to determine virus titer (mirusbio.com/flashbac).

For Research Use Only

*flash*BAC™ Systems and pOET vectors are sold by Mirus Bio through partnership with Oxford Expression Technologies, Oxford, UK.

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