BacPAK™ Baculovirus Expression System Protocol-At-A-Glance

Please read the User Manual for the BacPAK™ Baculovirus Expression System (Cat. Nos. 631402) before using this Protocol-At-A-Glance. **This abbreviated protocol is provided for your convenience, but is not intended for first-time users.**

I. Day 1–3: Preparation of Transfer Vectors

1. Insert target gene into the pBACPAK8 or pBACPAK9 transfer vector and prepare plasmid DNA.

II. Day 4: Cotransfection

1. Seed each dish with 1.0 x 10^6 cells in 1.5 ml of insect cell complete medium, supplemented with 10% FBS. Incubate in a humid plastic storage box at 27°C for 1–4 hr.
2. Wash cell monolayers twice with 2 ml of Grace's insect medium. Incubate in media at room temperature for 10–30 min.
3. Gently mix the following:

   500 ng pBacPAK8 or pBacPAK9 with insert gene

   5 µl BacPAK6 viral DNA (Bsu36I digest)

   Add sterile H_2O to a final volume of 96 µl
4. Add 4 µl of the Bacfectin reagent to DNAs and mix gently. Incubate at room temperature for 15 min.
5. Remove the medium from the cell monolayers and add 1.5 ml of Grace's insect medium.
6. Add the Bacfectin reagent-DNA mixture dropwise to the medium while gently swirling the dish to mix. Incubate at 27°C for 5 hr.
7. Add 1.5 ml of complete medium to each dish. Incubate at 27°C for 5 days.

III. Day 7: Plaque Assay

1. Transfer the cotransfection supernatant medium, which contains viruses produced by the transfected cells, to a sterile tube. (Cotransfection supernatant can be stored at 4°C for months.)
2. Seed 35-mm dishes with 1.5 x 10^6 cells per dish and incubate at 27°C for 1–4 hr.
3. Make serial dilutions of the cotransfection supernatant (100 µl into 900 µl of complete medium) to obtain final dilutions of 10^{-1}, 10^{-2}, and 10^{-3}.
4. Aspirate the medium. Infect dishes by gently adding 100 µl of the virus dilution to the center of the dishes. Incubate at room temperature for 1 hr on a level surface so the virus can infect the cells.
5. Melt 2% SeaPlaque agarose (Cambrex) in autoclaved H_2O, and cool to 37°C. Prewarm complete medium to 37°C. Add the warm complete medium to the agarose and mix. Keep at 37°C until Step 6 is finished.

   NOTE: To assay for recombinant viruses in the control cotransfection, use complete medium containing 300 µg/ml X-GLUC. (Final concentration in agarose overlay is 150 µg/ml.)
6. Remove the virus inoculum from the cells by tilting the dish and aspirating from the edge.
7. Gently add 1.5 ml of the agarose overlay to each dish, allowing the agarose to run down the side of the dish.
8. When the agarose overlay has set, add 1.5 ml of complete medium to each dish. Incubate dishes in a humid plastic storage box with a tight-fitting lid at 27°C for 7 days.

   NOTE: To assay for recombinant viruses in the control cotransfection, use complete medium containing 150 µg/ml X-GLUC.

IV. Day 11/12: Plaque Staining

1. Add 1 ml of 0.03% neutral red solution to the media in each of the dishes. Incubate at 27°C for 2–3 hr.
2. Aspirate off the stain, invert the dishes, and incubate overnight in the dark at room temperature to allow the plaques to clear and the blue color to develop on the positive control.

V. Day 12/13: Picking Plaques for Virus Propagation

1. Prepare a sterile microcentrifuge tube containing 0.5 ml of complete medium for each well-isolated plaque identified.
2. Pick plaques into sterile microcentrifuge tubes containing 500 µl of complete medium. Vortex and store at 4°C.

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