Certificate of Analysis

BacPAK™6 DNA (Bsu36 I digest)

Catalog No.	Amount	Lot Number
631401	5 transfections	Specified on product label.

Description

Baculovirus DNA specially designed and prepared to give a high proportion of recombinant viral expression vectors (Kitts and Possee 1993). The Bsu36 I-digested BacPAK6 DNA is ready-to-use and is sufficient for 5 transfections. The transfection reagent Bacfectin is also provided.

Virus Size

- 130 kb

Package Contents

- 25 µl BacPAK6 DNA (Bsu36 I digest)
- 25 µl Bacfectin

Storage Conditions

- Store at 4°C (Do not freeze.)
- Spin briefly to recover contents.

Shelf Life

- 1 year from date of receipt under proper storage conditions.

Storage Buffer

- 10 mM Tris-HCl (pH 8.0)
- 1 mM EDTA

Shipping Conditions

- Blue ice (4°C)

References

Kitts, P. A. & Possee, R. D. A method for producing recombinant baculovirus expression vectors at high frequency. *Biotechniques* **14,** 810–812+814+816 (1993).

Quality Control Data

BacPAK6 viral DNA (Bsu36 I digest) and pBacPAK8-GUS (a transfer vector containing the beta-glucuronidase gene) were cotransfected into IPLB-Sf21 cells following the recommended protocol. Progeny viruses were plaque-assayed (Kitts and Possee 1993) with the addition of X-Gluc (a chromogenic substrate for b-glucuronidase), to identify recombinant viruses, and in parallel with X-Gal to identify viruses generated from incompletely digested pBacPAK6 DNA.

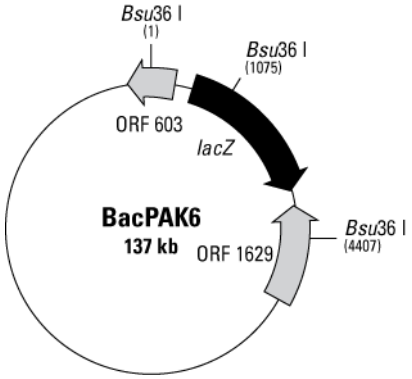
Takara Bio USA, Inc.
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: techUS@takarabio.com

United States/Canada 800.662.2566
Asia Pacific +1.650.919.7300
Europe +33.(0)1.3904.6880
Japan +81.(0)77.565.6999

(072417)

The percentage of blue plaques resulting from addition of X-Gluc was ≥70% of the total plaques. This is an underrepresentation of the actual percentage of viruses that have undergone the correct recombination, as blue color is extremely difficult to detect in smaller plaques.

The percentage of blue plaques resulting from addition of X-Gal was ≤1% of those observed for X-Gluc for the recombinant virus.



Restriction of BacPAK6 viral DNA with Bsu36 I removes a fragment that includes part of an essential gene (ORF1629). After cotransfection of insect cells with Bsu36 I-digested BacPAK6 viral DNA and transfer vector DNA, double recombination events that transfer the target gene from the transfer vector to the viral genome also restore the integrity of the essential gene. The unrecombined large Bsu36 I fragment of BacPAK6 is unable to produce viable viruses. This selection results in nearly 100% of the viral plaques containing recombinant viruses that have acquired the target gene (Kitts and Possee 1993). The sequences necessary to rescue Bsu36 I-digested BacPAK6 viral DNA are present in pBacPAK8, pBacPAK9, pBacPAK8-GUS, pAcUW31, and most other polyhedrin-based AcMNPV transfer vectors (including pVL941, pVL1392, pVL1393, pEV55, pAcYM1, and pAc373).

It is certified that this product meets the above specifications, as reviewed and approved by the Quality Department.

Generating recombinant viral expression vectors using BacPAK6 DNA

DNA from a transfer vector containing the target gene is transfected into *Spodoptera frugiperda* cells along with Bsu36 I-digested BacPAK6 viral DNA. *In vivo* homologous recombination between the plasmid and viral DNAs rescues the viral DNA and in the process transfers the target gene to the viral genome.

1. Prepare plasmid DNA from a transfer vector containing the target gene by CsCl isopycnic (density gradient) centrifugation or by alkaline lysis miniprep followed by purification with a CHROMA SPIN-400 Column.
2. Dilute the plasmid DNA to 100 ng/µl with TE [10 mM Tris-HCl (pH 8.0), 1 mM EDTA].
3. Seed two 35-mm tissue culture dishes with 1 x 106 exponentially growing Sf21 or Sf9 cells and incubate at 27°C for 1–4 hr.
4. Remove the old medium from the cells and add 2 ml BacPAK Grace's Basic Medium (Cat. No. 631404). Swirl gently, remove the medium again and add 2 ml BacPAK Grace's Basic Medium. Incubate at room temperature for 10–30 min while the Bacfectin-DNA mixture is prepared, as described in the following steps.

   NOTE: Transfection is inhibited by a component of serum; therefore, this washing step is necessary to replace the normal medium with serum-free medium before adding the Bacfectin-DNA mixture to the cells.

5. Make the following additions to two sterile polystyrene tubes:

	Cotransfection	Control
Sterile H_2O	86 µl	91 µl
Plasmid DNA (100 ng/µl)	5 µl	5 µl
BacPAK6 viral DNA (Bsu36 I digest)	5 µl	—

NOTES:

- Transfecting the plasmid DNA alone provides a control which will reveal any contamination in the reagents.
- Baculovirus DNA is large and is easily damaged by shearing, and BacPAK6 DNA will lose its infectivity if damaged. Therefore, the viral DNA should be handled with care throughout these procedures. For example, to mix solutions containing BacPAK6 DNA, gently flick the tube rather than vortexing it.

6. Add 4 µl of Bacfectin to the DNAs and mix gently. Incubate at room temperature for 15 min to allow the Bacfectin to form complexes with the DNA.
7. Meanwhile, remove the medium from the cell monolayers and add 1.5 ml BacPAK Grace's Basic Medium.
8. Add the Bacfectin-DNA mixture dropwise to the medium while gently swirling the dish to mix. Incubate at 27°C for 5 hr.
9. Add 1.5 ml BacPAK Complete Medium (Cat. No. 631403) or TNM-FH containing 10% fetal bovine serum and antibiotics to each dish. Incubate at 27°C.
10. Approximately 72 hr after addition of the Bacfectin DNA mixture to the cells, transfer the medium, which contains viruses produced by the transfected cells, to a sterile container and store at 4°C.
11. (optional) To obtain more virus, add 1.5 ml BacPAK Complete Medium or TNM-FH/FBS to each dish. Incubate at 27°C for another 2 days and harvest the medium as above.
12. Perform a plaque assay with the cotransfection supernatant at dilutions of 10^{-1}, 10^{-2}, and 10^{-3} to produce individual plaques.

    Usually the plaques produced from a BacPAK6 cotransfection are nearly all recombinant; however, it is wise to pick up to 10 well-isolated plaques, if possible. Small scale infections with a few (3–4) of these putative recombinant viruses can be performed to amplify the viruses and generate virus-infected cell proteins or DNA that can be used to confirm that one or more of the plaques contains a recombinant baculovirus. A confirmed recombinant virus can be further amplified to obtain a high titer working stock.

    Or, assume that most of the viruses in the cotransfection supernatant are recombinant and use this as the 1° stock of recombinant virus. This stock can be amplified and used to express protein. Such a stock will contain a mixture of viruses and its composition may change with repeated passage resulting in altered expression. This short cut can be used to quickly produce a few batches of protein but if many batches of protein are to be produced it is recommended that a clonal stock of virus is produced to ensure consistency.

Notice to Purchaser

BacPAK™6 DNA (Bsu36 I digest)

CATALOG NO.

631401

NOTICE TO PURCHASER:

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Takara Bio USA, Inc.
1290 Terra Bella Avenue, Mountain View, CA 94043, USA
U.S. Technical Support: techUS@takarabio.com

United States/Canada	Asia Pacific	Europe	Japan
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