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Product Information

DiamondBac™ Baculovirus DNA

Catalog Number **D6192**
Storage Temperature 2-8 °C (*Do not freeze*)

TECHNICAL BULLETIN

Product Description

DiamondBac™ Baculovirus DNA is a linearized modified baculoviral DNA derived from AcNMPV (*Autographa californica* nuclear multiple polyhedrosis virus[1]) that allows rapid and convenient generation of recombinant baculovirus clones. DiamondBac DNA contains a deletion within the gene ORF1629 that is essential for viral replication.[2] Multiple Bsu36 I restriction sites were engineered near ORF1629 to ensure complete linearization of the parental viral genome and deletion of the ORF1629 sequences. Therefore, intact parental viral DNA is eliminated and recombination efficiencies approach 100% when the missing ORF1629 sequences are supplied by the appropriate transfer vector. Most transfer vectors that contain sequences for recombination at the polyhedrin locus are compatible with DiamondBac. Recombinant viruses made with DiamondBac will also contain a protein disulfide isomerase (PDI) gene inserted at the p10 locus of the virus. PDI acts as a protein chaperone to aid in proper disulfide bond formation and overall folding of the desired recombinant protein following infection of insect cells.[3] In addition, cells infected with a recombinant virus derived from DiamondBac have higher cell viabilities throughout the course of infection as compared to wild-type virus due to the effect of the deleted p10 gene. These modifications result in up to a 10-fold increase in overall protein production for many recombinant proteins.[4]

Components/Reagents

DiamondBac baculovirus DNA is packaged with sufficient linearized virus for 15-25 co-transfections.

- DiamondBac Baculovirus DNA, 5 µg at 0.1µg/µl

Precautions and Disclaimer

DiamondBac Baculovirus DNA is for R&D use only, not for drug, household, or other uses. Consult the MSDS for information regarding hazards and safe handling procedures.

Storage/Stability

All components are guaranteed to be stable for at least six months after receipt when stored and handled properly at 2-8 °C. **Do not freeze the viral DNA.** Excessive pipetting may cause shearing of the high molecular weight DNA.

Reagents and Equipment Required but Not Provided

(Sigma Product Numbers have been given where appropriate)

- Purified baculovirus transfer vector containing the gene of interest (~ 1 µg/µl in 1X Tris-EDTA buffer)
- *Sf* 9 or *Sf* 21 insect cells in log phase growth
- EX-CELL® TiterHigh™ Medium, Catalog No. I5408
- Fetal Bovine Serum, Catalog No. F0643
- ESCORT™ IV Transfection Reagent, Catalog No. L3287
- 27 °C incubator
- 60 mm sterile tissue culture dishes
- polystyrene tubes (sterile)

Procedure

<u>Transfection Protocol for Insect Cells using DiamondBac Viral DNA</u>

1. Seed 60 mm tissue-culture dishes with approx. 8 x 10^5 *Sf* 9 or *Sf* 21 cells per plate. Cells should be in log phase of growth and ≥ 95% viable.
2. Incubate cells at 27°C for 30 minutes to 1 hour to allow the cells to attach to the dishes.
3. To prepare each transfection mixture, first mix 5 µl of ESCORT IV* with 95 µl of serum-free TiterHigh insect medium in a sterile polystyrene tube. In another sterile tube, add 0.1 µg of DiamondBac viral DNA (0.1µg/µl) and 1µg of a recombinant transfer vector (1 µg/µl) to 98 µl of serum-free TiterHigh insect medium.

(*Note: See ESCORT IV technical bulletin for complete instructions about optimizing transfection conditions such as the ratio of DNA to lipid).

4. Add the diluted DNA mixture to the diluted ESCORT IV and swirl gently.
5. Incubate the transfection mixture at room temperature for 15 to 45 minutes.
6. Aspirate the medium from the attached cells and wash the monolayers with serum-free medium. Do not let the cells dry out.
7. Add 0.8 ml of fresh serum-free medium to cells. Then, add the 0.2 ml of the transfection mixture and incubate the cells with the transfection mixture for four to five hours at 27 °C.
8. Aspirate the transfection mixture from the cells. Wash cells once with 2 ml of serum-free medium. Replenish the cells with fresh medium (either serum-free or serum-supplemented).
9. Incubate for 5 to 6 days at 27 °C and monitor via morphology for signs of viral infection.
10. Collect the supernatant of all samples via centrifugation in sterile tubes. Store samples at 4 °C. Virus titers at this stage will be very low (approx. 1 x 10^3 to 1 x 10^5 PFU/ml)
11. Use transfection supernatant samples to infect fresh cells in order to amplify the virus for obtaining high titer virus stocks (>1 x 10^8 PFU/ml). Optionally, lyse the transfected cells and check for expression of your protein of interest.

References

1. O'Reilly, D.R., Miller, L.K., and Luckow, V.A. (1992). *Baculovirus Expression Vectors: A Laboratory Manual* (New York, N.Y.: W. H. Freeman and Company).
2. Kitts, P.A., Ayres, M.D., and Possee, Linearization of baculovirus DNA enhances the recovery of recombinant virus expression vectors. *Nucleic Acids Research* **18**:5667-5672 (1990).
3. Tsu-An Hsu, Sarah Watson, Joseph J. Eiden and Michael J. Betenbaugh, Rescue of Immunoglobulins from Insolubility Is Facilitated by PDI in the Baculovirus Expression System *Protein Expression and Purification,* **7**, Issue 3,: 281-288 (1996).
4. Williams, G.V., Rohel, D.Z., Kuzio, J., and Faulkner, P., A cytopathological investigation of *Autographa californica* nuclear polyhedrosis virus p10 gene function using insertion deletion mutants. *J. Gen. Virol.* **70**: 187-202 (1989).

Troubleshooting Guide

Problem	Cause	Solution
Failed transfection/no recombinant virus	Impurities in plasmid DNA preparation	Include a wash step in procedure that uses 70% ethanol to remove residual salts; Check plasmid DNA on gel to ensure integrity
	Poor cell viability	Cells must be in log phase and 95-98% viable for successful transfection
	Transfection variables not optimized	Be sure cell density and DNA to lipid ratio is correct
	Lack of proper recombination sequences on transfer vector	Verify the transfer vector contains ORF1629 and ORF603 sequences.
Contamination	Impure plasmid DNA or poor aseptic technique	Include a plasmid DNA control. Use good aseptic technique at all times. An antibiotic such as gentamicin (Product No. G 1397) at 50 ug/ml may be included in the medium.

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