Technical Data Sheet

pVL1392-XylE Baculovirus Control Vector

Product Information

Material Number:	**554807**
Size:	5 µg
Storage Buffer:	10 mM Tris-HCl, pH 7.5; 1 mM EDTA

Description

The pVL1392-XylE Control Vector is a purified Baculovirus transfer vector which can be used as a positive control in co-transfection with Pharmingen's BaculoGold™ Baculovirus DNA, Cat. No. 554739. In this vector, the Pseudomonas putrida gene XylE was cloned into the pVL1392 Baculovirus Transfer Vector. Co-transfection with BaculoGold™ DNA will generate recombinant baculoviruses that express the XylE protein which runs as a 35 kD protein on SDS-PAGE. Infected insect cells producing the XylE protein will turn yellow in the presence of catechol (500 µM catechol, 50 µM bisulfate).

Preparation and Storage

Store undiluted at -20° C.

The plasmid DNA was purified using an anion-exchange resin and dissolved in TE buffer, 5 µg/50µl.

Application Notes

Recommended Assay Procedure:

For expression of the XylE protein, perform a co-transfection (please refer to accompanying protocol) of the pVL1392XylE vector and linearized baculovirus DNA (BaculoGold™ DNA Cat. No. 554739) into a susceptible insect cell line (*Sf*9 or *Sf*21)*. For detection of the XylE protein, prepare a solution of 500 µM catechol, 50 µM bisulfate in sterile water. When detecting protein generated by infection of monolayer insect cell cultures, add 100 µl of the catechol solution. To detect plaques of recombinant XylE virus, add enough catechol solution to cover the entire surface of the agarose. Infected cells should turn bright yellow in less than 10 minutes. It is helpful to compare infected cells to a negative control of uninfected cells.

Reference: *Please visit us at www.bdbiosciences.com to view or request a Baculovirus Expression Vector System Manual.

GENERATION OF RECOMBINANT BACULOVIRUS BY CO-TRANSFECTION

MATERIALS NEEDED

6 X 10e6 *Sf*9 cells in log phase
3 tissue culture plates (60 mm)
15 ml TNM-FH insect medium (Cat. No. 554760)
1.0 µg BacuolGold™ baculovirus DNA (Cat. No. 554739)
2 µg recombinant baculovirus transfer vector DNA containing your gene
2 µg pVL1392-XylE Control Vector (Cat. No. 554807)
2 ml Transfection Buffer A (Grace's medium containing 10% heat-inactivated fetal calf serum, pH 6.0-6.2)
2 ml Transfection Buffer B (25 mM HEPES pH 7.1, 125 mM CaCl2, 140 mM NaCl)

PREPARATION OF TRANSFER VECTOR CONTAINING GENE OF INTEREST

For co-transfection, prepare at least 10 µg of highly purified plasmid DNA. Impure preparations of plasmid DNA are toxic to *Sf*9 cells, and may cause cells to lyse shortly after transfection. The result is an apparently lower viral titer. Miniprep DNA may be prepared using an isolation filter or silicon bead matrix, but DNA must be sterile. Phenol/chloroform extraction and ethanol precipitation may not remove contaminants which are toxic to the cell. At 24 hr post-transfection, Sf9/Sf21 cell viability should be greater than 97%.

PROTOCOL FOR CALCIUM PHOSPHATE-MEDIATED CO-TRANSFECTION

1. Prepare three tissue culture plates; one each for the co-transfection, positive co-transfection control (pVL1392-XylE, Cat. No. 554807), and negative control (cells only). Seed 2x10e6 Sf9 cells onto each 60 mm tissue culture plate. Initial cell density should be approximately 50-70% confluent. Cell attachment should be done on a flat and even surface, allowing the cells to attach firmly, usually about 5 min. If cells don't attach after that time, they are either not healthy or the wrong plates (e.g., non-coated petri dishes) have been used.

NOTE: A fourth tissue culture plate may be seeded with Sf9 cells for infection with wildtype AcNPV virus as a positve control for infection.

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2. While cells are attaching to the plate, mix 0.5 µg BaculoGold™ DNA and 2-5 µg recombinant Baculovirus Transfer Vector, containing your gene, in a microcentrifuge tube. Mix well, by gentle vortexing or by flicking the tube. Let mixture sit for five minutes before adding 1 ml of Transfection Buffer B.
3. Prepare co-transfection positive control by mixing 0.5 µg BaculoGold™ DNA and 2 µg pVL1392-XylE Control Transfer Vector DNA in a microcentrifuge tube. Mix well, by gentle vortexing or by flicking the tube. Let mixture sit for five minutes before adding 1 ml of Transfection Buffer B.
4. Label the first culture plate as the co-transfection plate. Aspirate the old medium from the plate and replace it with 1 ml of Transfection Buffer A. Make sure that the entire surface of plate is covered, to prevent the cells from drying out.
5. Label the second plate as the co-transfection positive control. Aspirate the old medium from the plate and replace it with 1 ml of Transfection Buffer A, as in Step 4.
6. Label the third culture plate as a negative control. Aspirate old medium, replace with 3 ml fresh TNM-FH medium, and add nothing to this plate.
7. Add the 1 ml of Transfection Buffer B/DNA solution, from Step 2, drop-by-drop to the co-transfection plate. After every three to five drops, gently rock the plate back and forth to mix the drops with the medium. During this procedure, a fine calcium phosphate/DNA precipitate should form. This precipitate is characterized by a fine white milky color.
8. Add the 1 ml of Transfection Buffer B/XylE Positive Control DNA solution, from Step 3, drop-by-drop to the co-transfection control plate, repeating the procedure in Step 7.
9. Incubate all three plates at 27°C for 4 hours.
10. After 4 hours, remove the medium from the co-transfection plate and the co-transfection positive control plate. Add 3 ml fresh TNM-FH medium and rock the plate back and forth several times before once again removing all the medium. Add 3 ml of fresh TNM-FH medium and incubate the plates at 27°C for 4-5 days. It is not necessary to change the media of the negative control.
11. After 4 days, check the three plates for signs of infection. Compare the negative and positive controls to the co-transfection plate. Infected cells will appear much larger than uninfected ones, have enlarged nuclei, stop dividing, and will float in the medium.
12. After 5 days, collect the supernatant of the co-transfection and co-transfection positive control plates. Assess co-transfection efficiency by end-point dilution assay or identify recombinant viruses by plaque screening. The transfection supernatant should be amplified to produce a high-titer virus stock, used for production of the recombinant protein by infection of insect cells. Alternatively, a single recombinant virus, obtained by plaque purification, may be used for virus amplification. To check the expression of your protein of interest, lyse the transfected cells or use an aliquot of the supernatant (depending whether the recombinant protein is secreted or not). Cells expressing the XylE protein can be assayed by adding 100 µl of a 500 µM catechol, 50 µM sodium bisulfate solution. Infected cells will turn bright yellow in approximately 5 min.

TROUBLE SHOOTING

1. There is no precipitate.
   Check pH of Transfection Buffer B. It should be pH 7.1 plus or minus 0.05.
   Note: The precipitate may not be identifiable under a microscope. A successful precipitation is characterized by a milky white color which is visible to the naked eye.
2. All the cells die within a day after transfection.
   Check purity of your plasmid DNA. Dirty DNA can cause cell death. Perform a mock co-transfection, without linearized baculovirus DNA, of 1) your recombinant transfer vector and 2) the non-recombinant transfer vector, and compare the health of the cells beginning at 4 h post transfection.
3. When using lipofectin.
   Check that you did not use media with fetal calf serum. Use serum-free medium for lipofection.

Suggested Companion Products

Catalog Number	Name	Size	Clone
554806	Transfection Buffer A and B Set	5 transfections	(none)
554760	TNM-FH Insect Medium	1 liter	(none)
554739	Linearized Baculovirus DNA	5 transfections	(none)

Product Notices

1. Please refer to www.bdbiosciences.com/pharmingen/protocols for technical protocols.