

## RetroPack<sup>™</sup> PT67 Cell Line

**Catalog No.** 631510

Lot Number Specified on product label.

## Description

An NIH 3T3-derived cell line designed for the production of infectious, replication-incompetent virus. PT67 contains the Moloney murine leukemia virus (MoMuLV) gag, pol, and env (10A1-derived) genes. Transfection with a retroviral vector containing the retroviral packaging signal and a target gene allows production of replication-incompetent virus (1, 2).

### **Package Contents**

• 1 ml RetroPack PT67 Cell Line (2 x 10<sup>6</sup> cells/tube)

#### **Storage Conditions**

• Store cells in liquid nitrogen (-196°C) or in a -150°C freezer

#### Shelf Life

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

#### Storage Medium

• Cell Freezing Medium-DMSO 1x (Sigma-Aldrich Co., Cat. No. C6164)

### **Shipping Conditions**

• Dry ice  $(-70^{\circ}C)$ 

#### **Product Documents**

Documents for Clontech® products are available for download at <u>www.clontech.com/manuals</u> The following documents apply to this product:

• Retroviral Gene Transfer and Expression User Manual (PT3132-1)

### **Cell Type Information**

NIH 3T3-derived cell line stably expressing the retroviral gag and pol genes, cointroduced with the thymidine kinase gene; and the 10A1 envelope gene, cointroduced with the dihydrofolate reductase gene.

## **Recommended Cell Culture Medium**

90% DMEM; 10% fetal bovine serum; 4 mM L-glutamine; 100 units/ml penicillin G sodium; 100 µg/ml streptomycin sulfate; and 1 mM sodium pyruvate.

# Certificate of Analysis

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## **Recommendations for Thawing Frozen Cells**

We recommend initiating the culture as soon as possible upon receipt. If the cells cannot be thawed and cultured immediately upon receipt, the vial should be held at temperatures below  $-79^{\circ}$ C, preferably in liquid nitrogen vapor.

- 1. Thaw rapidly by placing tube of frozen culture in a 37°C water bath.
- 2. To reduce osmotic shock, dilute the cell suspension in 1 ml of complete growth medium and transfer it to a sterile 15 ml tube.
- 3. Add 5 ml of complete medium and mix.
- 4. Add an additional 5 ml of complete medium and mix gently.
- 5. Pellet the cells for 10 min at 125 x g. Discard supernatant.
- 6. Resuspend the pellet in 10 ml of complete growth medium and seed culture into a flask or culture dish.

### References

- 1. Coffin, J., et al. (1996) Retroviruses (CSHL Press).
- 2. Ausubel, F. M., et al. (1996) Current Protocols in Molecular Biology (John Wiley & Sons, NY) Supp. 36, Section III.

## **Quality Control Data**

### **Functional Tests**

The RetroPack PT67 cell line was functionally tested for its ability to produce infectious virus. RetroPack PT67 cells were transfected with pSIREN-RetroQ-ZsGreen RetroviralVector. After 48 hours, virus was collected and overlaid on NIH 3T3 cells. After three days, the NIH 3T3 cells were analyzed by flow cytometry to confirm retroviral infection. The titer of virus produced by RetroPack PT67 cells was at least 10<sup>5</sup> cfu/ml.

#### Safety Tests

This cell line was tested to ensure the absence of replication-competent retroviral particles. Culture medium from RetroPack PT67 cells was overlaid on NIH 3T3-pLAPSN stable cells. After 2–3 days in culture, medium from these cells was overlaid on fresh NIH 3T3 cells. Staining of these cells revealed the absence of alkaline phosphatase expression, thus confirming the absence of retroviral infection.

#### Mycoplasma Contamination Test

This lot of the RetroPack PT67 Cell Line was tested and found to be free of Mycoplasma contamination.

**NOTE:** The viral supernatants produced by these retroviral systems could, depending on your cloned insert, contain potentially hazardous recombinant virus. Due caution must be exercised in the production and handling of recombinant retrovirus. Appropriate NIH, regional, and institutional guidelines apply. The User Manual contains other general information and precautions.



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#### **STATEMENT 82**

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