Certificate of Analysis

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PRODUCT: Retro-X™ System

CATALOG No. 631508

LOT NUMBER
Specified on product label.

STORAGE CONDITIONS
Plasmids and Primers
- Store at –20°C.
- Spin briefly to recover contents.
- Avoid repeated freeze/thaw cycles.

RetroPack™ PT67 Packaging Cell Line
Liquid nitrogen (–196°C)

PLASMID STORAGE BUFFER
10 mM Tris-HCl (pH 8.0)
1 mM EDTA (pH 8.0)

CELL STORAGE MEDIUM
70% FBS + 20% DMEM + 10% DMSO

SHELF LIFE
1 year from date of receipt under proper storage conditions.

SHIPPING CONDITIONS
Dry ice (–70°C)

FOR RESEARCH USE ONLY

DESCRIPTION
A complete retroviral gene expression system for introducing stable heritable genetic material into mammalian cell lines. This system can be used to infect dividing cells from a broad range of mammalian cell lines.

CELL TYPE AND MEDIA INFORMATION
See back of page.

PACKAGE CONTENTS
- 20 µg pLNCX2 Retroviral Vector (500 ng/µl)
- 20 µg pLXSN Retroviral Vector (500 ng/µl)
- 20 µg pLAPSN Retroviral Vector (500 ng/µl)
- 1 ml RetroPack™ PT67 Cell Line (2 x 10^6 cells/tube)
- 100 µl pLNCX Seq/PCR Primers (20 µM)
- 100 µl pLXSN Seq/PCR Primers (20 µM)
- User Manual (PT3132-1)
- Vector Information Packets (PT3297-5, PT3135-5, & PT3134-5)

QUALITY CONTROL DATA

1. Plasmid QC Results

Identity and purity

The identity of each plasmid was verified by electrophoresis on an agarose/EtBr gel after digestion with the indicated enzymes. The purity of each plasmid was also checked by determining the A_{260}/A_{280}.

Vector	Plasmid Size (kb)	Enzyme	Fragment Size (kb)
pLNCX2	6.1	*Eco R* I/*Xba* I	2.6, 1.9, and 1.6
pLXSN	5.9	*Sac* I/*Hind* III	3.1, 1.4, and 1.3
pLAPSN	7.9	*Bam H* I	5.9, 1.7, and 0.3

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.

APPROVED BY: _____

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QUALITY CONTROL DATA

2. RetroPack™ PT67 Packaging Cell Line

Functional testing

The RetroPack™ PT67 cell line was functionally tested for its ability to produce infectious virus. RetroPack PT67 cells were transfected with pSIREN-RetroQ-ZsGreen Retroviral Vector. 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 10^5 cfu/ml.

Safety testing

This cell line was tested to ensure the absence of production of replication-competent retrovirus. 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 then overlaid on fresh NIH 3T3 cells. Staining of these cells indicated an absence of alkaline phosphatase expression, thus confirming the absence of retroviral infection.

Mycoplasma testing

This lot of the RetroPack PT67 cell line was tested and found to be free of mycoplasma contamination.

3. Primers

The primers were tested by sequencing using a positive control template.

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

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

RECOMMENDATIONS FOR THAWING FROZEN CELLS

Initiate culture as soon as possible upon receipt.

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 complete growth medium and transfer it to a larger 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 complete growth medium and seed culture into a flask or culture dish.