

AmphoPack-293 Cell Line

Catalog No.

631505

Lot Number

Specified on product label.

Description

A human embryonic kidney (HEK 293)-derived cell line. This cell line is designed for rapid production of high-titer replication-incompetent retrovirus by transient or stable transfections. The AmphoPack-293 Cell Line was developed using bleomycin and puromycin resistance genes to stably introduce the viral *gag-pol* and *env* genes, respectively. Therefore, stable cell lines can be developed using neomycin and hygromycin selectable markers. Virus produced by AmphoPack-293 cells can infect a broad range of mammalian cells.

Package Contents

- 1 ml AmphoPack-293 Cell Line (2×10^6 cells/ml)

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°C)

Product Documents

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

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

Cell Type Information

HEK-derived cell line transformed with adenovirus type 5 DNA (HEK 293). The viral *gag*, *pol*, and 4070A *env* genes were cointroduced into the AmphoPack-293 genome using bleomycin and puromycin resistance genes.

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.

Clontech Laboratories, Inc.

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Recommendations for Thawing Frozen Cells

Initiate culture as soon as possible upon receipt. AmphoPack-293 cells require collagen-coated plates for efficient recovery of frozen stocks.

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 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 **collagen-coated** flask or culture dish.

NOTE: Culture vessels coated with compounds other than collagen may provide suitable growth substrates for AmphoPack-293 cells; however, only collagen-coated plates have been tested at Clontech Laboratories, Inc. We recommend the BD Biosciences - Discovery Labware Falcon BioCoat Collagen I Cellware for culturing AmphoPack-293 cells. The cells may be cultured on regular flasks/dishes (i.e., non-coated flasks/dishes) after recovery; however, if adherence is poor, we recommend collagen-coated vessels for all culturing purposes, including viral packaging.

Quality Control Data

Functional Tests

The AmphoPack-293 Cell Line was functionally tested for its ability to produce infectious virus. AmphoPack-293 cells were transfected with a retroviral vector expressing ZsGreen1. 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 AmphoPack-293 cells was at least 10^5 cfu/ml.

Safety Tests

This cell line was tested to ensure the absence of replication-competent virus. Culture medium from AmphoPack-293 cells was overlaid on NIH 3T3 pLAPSN stable cells. After 2–3 days in culture, medium from the NIH 3T3 cells were 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 Contamination Test

This lot of the AmphoPack-293 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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