

EcoPack™ 2-293 Cell Line

Catalog No.

631507

Lot Number

Specified on product label.

Description

A cell line derived from human embryonic kidney (HEK 293). The cells are designed for rapid production of high-titer Moloney-based retroviral stocks by transient transfection, and are intended for use with retroviral vectors and related technology for functional expression analysis. Virus produced by these cells can infect mouse and rat cells.

Package Contents

- 1 ml EcoPack™ 2-293 Cell Line (2×10^6 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°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 cells express the MMLV Gag, Pol, and Env proteins.

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

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°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 $125 \times g$. Discard supernatant.
6. Resuspend the pellet in 10 ml of 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 EcoPack 2-293 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 EcoPack 2-293 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 EcoPack 2-293 cell line was functionally tested for its ability to produce infectious virus. EcoPack 2-293 cells were transfected with RNAi-Ready pSIREN-RetroQ-ZsGreen Vector (Cat. No. 632445). After 48 hr, virus was collected and overlaid on NIH 3T3 cells. After 3 days, the NIH 3T3 cells were analyzed by flow cytometry to confirm retroviral infection.

The titer of virus produced by EcoPack 2-293 cells was at least 10^5 cfu/ml.

Safety Tests

This cell line was tested to ensure the absence of replication-competent retroviral particles. Culture medium from EcoPack 2-293 cells was overlaid on NIH 3T3-pLAPSN stable cells. pLAPSN is a retroviral vector expressing alkaline phosphatase. After 2–3 days in culture, medium from these cells was then 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 EcoPack 2-293 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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