

Platinum-E Retroviral Packaging Cell Line, Ecotropic

CATALOG NUMBER: RV-101

STORAGE: Liquid nitrogen

Note: For best results begin culture of cells immediately upon receipt. If this is not possible, store at -80°C until first culture. Store subsequent cultured cells long term in liquid nitrogen.

QUANTITY & CONCENTRATION: 1.0 mL, $>3 \times 10^6$ cells/mL in DMEM, 20% FCS, and 10% DMSO

Background

Retroviruses are efficient tools for delivering heritable genes into the genome of dividing cells. However, conventional NIH-3T3 based retroviral packaging cell lines have limited stability and produce low viral yields, mainly due to poor expression level of the retroviral structure proteins (gag, pol, env) in the packaging cells.

The Platinum-E (Plat-E) Cell Line, a potent retrovirus packaging cell line based on the 293T cell line, was generated using novel packaging constructs with an EF1 α promoter to ensure longer stability and high-yield retroviral structure protein expression (gag, pol, ecotropic env). Plat-E cells can be kept in good condition in for at least 4 months in the presence of drug selection, and can produce retroviruses with an average titer of 1×10^7 infectious units/mL by transient transfection. In addition, replication competent retroviruses (RCR) are virtually nonexistent because only coding sequences of viral structural genes are used, avoiding any unnecessary retroviral sequences.

The Plat-E cell line is designed for rapid, transient production of high-titer ecotropic retrovirus.

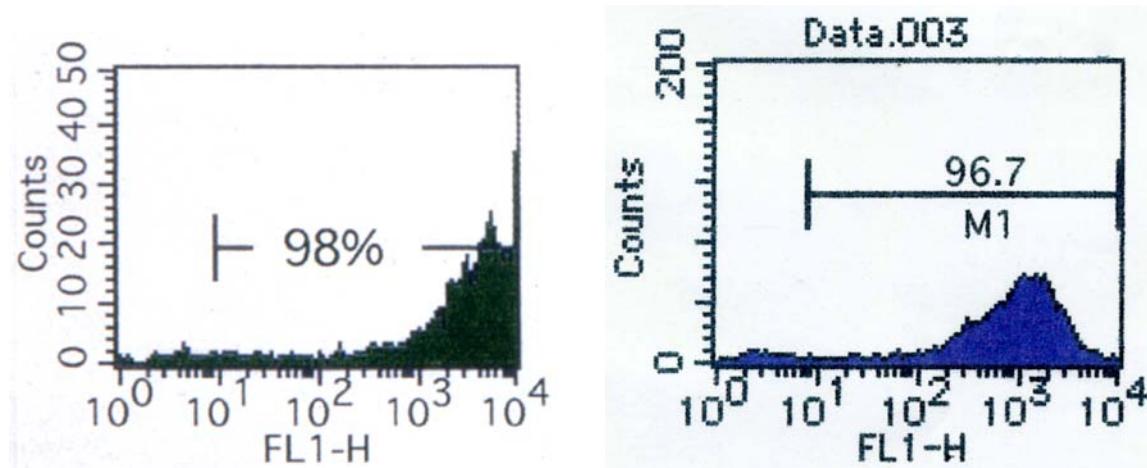


Figure 1. NIH3T3 cells (left) and mouse ProB Ba/F3 cells (right) were infected with GFP retrovirus supernatant produced in Plat-E cells after transfection with pMX-GFP.

Medium

1. Culture Medium: DMEM, 10% fetal calf serum (FCS), 1 μ g/mL puromycin, 10 μ g/mL blasticidin, penicillin and streptomycin
2. Freeze Medium: 70% DMEM, 20% FBS, 10% DMSO

Methods

I. Establishing Plat-E Cultures from Frozen Cells

1. After quickly thawing the cells in a 37°C water bath, immediately transfer the thawed cell suspension into a 15 mL tube containing 10 mL of culture medium.
2. Centrifuge the tube for 5 min at 1300 to 1500 rpm.
3. Discard the supernatant and break the cell pellet by finger tapping.
4. Add a few drops of culture medium with gentle shaking and finger tap the tube a few times.
5. Add 2 mL of culture medium to the tube and gently pipet the cell suspension up and down twice.
6. Transfer the cell suspension to a 10 cm culture dish (Falcon® #3003 works well) containing 8 mL of culture medium.
7. Swirl the culture plate well to mix the cells, then incubate the cells for three days before expansion.

Important Notes:

- ***Don't change the culture medium during the first three days. It is normal to see some cells floating after the first 24 hours.***
- ***Don't culture cells to complete confluence. Split cells 4X to 6X every two to three days when the culture reaches 70-90% confluence.***

II. Splitting the Cells

Note: Avoid forming bubbles as much as possible during this procedure.

1. Wash cells once with PBS.
2. Add 4 mL of 0.05% Trypsin/0.5 mM EDTA solution to a 10 cm dish and incubate at 37°C for 3-5 min.
3. Remove the cells from the dish surface by tapping the rim of the culture dish.
4. Transfer 10 mL of the culture medium to a 50 mL tube.
5. Using the same pipette with some residual culture medium, wash the dish surface gently three times in 4 mL of the Trypsin/EDTA solution.
6. Gently pipette the cell suspension up and down 7 times and transfer the cell suspension into the 50 mL tube containing 10 mL medium from step 4.
7. Centrifuge the cells for 5 min at 1300-1500 rpm.
8. Discard the supernatant and break the cell pellet by finger tapping.
9. Add a few drops of culture medium with gentle shaking and finger tap the tube a few times.
10. Add 5 mL of culture medium and gently pipet the cell suspension up and down twice.
11. Add 15 mL of culture medium, then count and seed the cells. Typically 10^7 cells can be harvested from one 10 cm culture dish.

Transfection

1. Seed 2×10^6 cells in a 60 mm culture dish without antibiotics including puromycin and blasticidin one day before transfection.

2. After 16 to 24 hours, start transfection when the culture becomes 70-80% confluent.
Note: We suggest transfecting cells with FuGENE® Transfection Reagent (Promega) or Lipofectamine™ Plus (Invitrogen). For example, 3 µg retroviral expression plasmid is mixed with 9 µL FuGENE® Transfection Reagent according to the manufacturer's recommendation. The mixed DNA- FuGENE® complex is added by dropwise into the culture media.
3. Harvest retroviral supernatant 48 hours after transfection.

References

1. Morita, S., Kojim, T., and Kitamura, T. (2000) *Gene Therapy* 7: 1063-1066.
2. Takahashi, K. and Yamanaka, S. (2006) *Cell* **126**: 663-676.

Recent Product Citations

1. Pagnon, J. et al. (2012). Identification and Functional Characterization of Protein Kinase A Phosphorylation Sites in the Major Lipolytic Protein, Adipose Triglyceride Lipase. *Endocrinology*. **153**:4278-4289.
2. Seo, W. et al. (2012). Runx1–Cbf β Facilitates Early B Lymphocyte Development by Regulating Expression of Ebf1. *J. Exp. Med.* **209**: 1255-1262.
3. Anantpadma, M and Vrati S.(2012) siRNA-Mediated Suppression of Japanese Encephalitis Virus Replication in Cultured Cells and Mice. *J.Antimicrob Chemother.* **67**:444-451.
4. Lahti, A.L. et al. (2012). Model for Long QT Syndrome Type 2 Using Human iPS Cells Demonstrates Arrhythmogenic Characteristics in Cell Culture. *Dis. Model. Mech.* **5**:220-230.
5. Tep, C. et al.(2012). Brain-derived Neurotrophic Factor (BDNF) Induces Polarized Signaling of Small GTPase (Rac1) Protein at the Onset of Schwann Cell Myelination through Partitioning-Defective 3 (Par3) Protein. *J.Biol Chem.* **215**:374-383.
6. Anantpadma, M and Vrati S.(2012) siRNA-Mediated Suppression of Japanese Encephalitis Virus Replication in Cultured Cells and Mice. *J.Antimicrob Chemother.* **67**:444-451.
7. Huang, H.P. et al. (2011). Epithelial Cell Adhesion Molecule (EpCAM) Complex Proteins Promote Transcription Factor-mediated Pluripotency Reprogramming. *J. Biol. Chem.* **286**:33520-33532.
8. Braunstein, M. et al. (2010). HEBALt Enhances the T-cell Potential of Fetal Myeloid-biased Precursors. *Int. Immunol.* **22**:963-972.
9. Lee, J-H. et al. (2010). Epigallocatechin-3-Gallate Inhibits Osteoclastogenesis by Down-Regulating c-fos Expression and Suppressing the Nuclear Factor k-B signal. *Mol Pharmacol.* **77**:17-25.
10. Li, W. et al. (2009). Rb/Cdk2/Cdk4 Triple Mutant Mice Elicit an Alternative Mechanism for Regulation of the G1/S Transition. *PNAS* **106**:486-491.
11. Cui, G. et al. (2009). Berberine Differentially Modulates the Activities of ERK, p38 MAPK, and JNK to Suppress Th17 and Th1 T Cell Differentiation in Type 1 Diabetic Mice. *J. Biol. Chem.* **284**:28420-28429.
12. Hinrichs, C.S. et al. (2009). Adoptively Transferred Effector Cells Derived from Naive Rather than Central Memory CD8+ Cells Mediate Superior Antitumor Immunity. *PNAS* **106**:17469-17474.
13. Rice, K.L. et al. (2009). Comprehensive Genetic Screens Identify a Role for PLZR-RARalpha as a Positive Regulator of Cell Proliferation via Direct Regulation of c-myc. *Blood* **114**:5499-5511.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

License Information

This licensed product is intended for ACADEMIC, GOVERNMENT AND NON-PROFIT RESEARCH USE ONLY; not for use in diagnostic or therapeutic procedures. The product may not be transferred, sold, or otherwise provided to another laboratory except by an authorized distributor of Cell Biolabs, Inc.

Use of this product by Biotechnology and Pharmaceutical companies requires a license for all fields of use including research. Please contact:

Director of Business Development
Cell Biolabs, Inc.
busdev@cellbiolabs.com

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2008-2012: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.