Product Data Sheet

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 -80ºC until first culture. Store subsequent cultured cells long term in liquid nitrogen.

QUANTITY & CONCENTRATION: 1.0 mL, >3 X 10^6cells/mL in DMEM,20% FBS, 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 EF1α 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 for at least 4 months in the presence of drug selection, and can produce retroviruses with an average titer of 1 x 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.

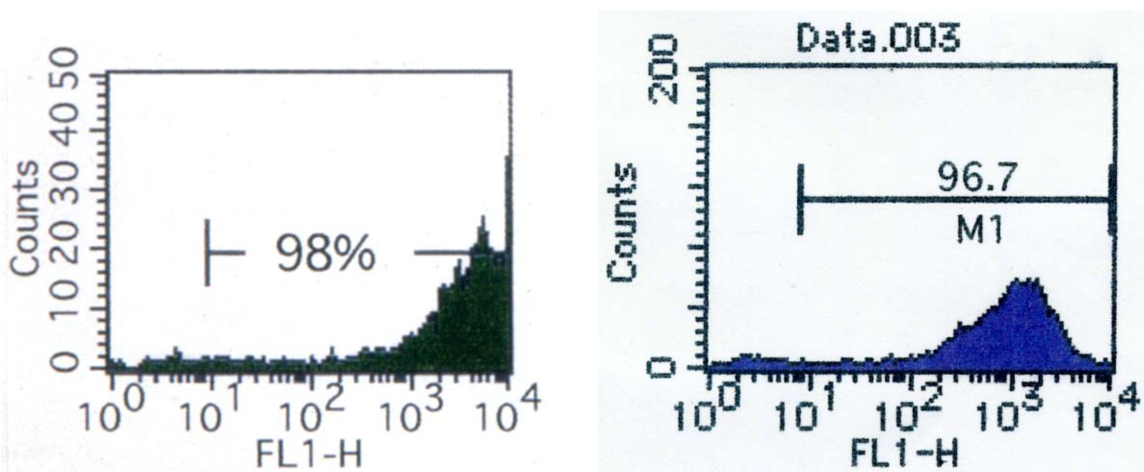

Figure 1. NIH3T3 cells (left) and mouse ProB Ba/F3 cells (right) were infected with GFP retrovirus supernatant produced in Plat-E cells after transfection with pMX-GFP.

Medium

1. Culture Medium: DMEM, 10% fetal bovine serum (FBS), 1 µg/mL puromycin, 10 µg/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 37ºC 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 confluency. Split cells 4X to 6X every two to three days when the culture reaches 70-90% confluency.***

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 37ºC 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 theTrypsin/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 x 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

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