

PLAT-E Cells | 305855

Informações gerais

Description

Plat-E (Platinum-E) is a retrovirus packaging cell line engineered from the human embryonic kidney 293T cell background. It was developed to provide a stable and efficient system for transient production of high-titer, ecotropic retroviruses. The cell line was constructed using novel packaging constructs in which the expression of viral structural genes - gag-pol and env - is driven by the human EF1 α promoter, which is substantially more potent in 293T cells than the conventional MuLV long terminal repeat (LTR) promoter. This design ensures robust transcriptional activity and supports high-level production of viral components necessary for efficient retrovirus assembly and packaging.

Plat-E cells were generated through sequential stable transfection of pEnv-IRES-puro and pGag-pol-IRES-bsd constructs, which link the viral genes to antibiotic resistance markers via internal ribosome entry sites (IRES). This configuration guarantees that only cells expressing the essential viral genes also gain antibiotic resistance, allowing for selection of high-expressing subclones. The resulting Plat-E line consistently produces retroviruses with titers up to 1×10^7 infectious units per milliliter for at least four months when cultured under dual selection with puromycin and blasticidin. Northern blot, reverse transcriptase activity, and flow cytometry analyses confirmed that Plat-E exhibits significantly higher gag-pol and env expression than predecessor packaging lines such as Bosc23 and Phoenix-E.

Plat-E's architecture minimizes the risk of generating replication-competent retrovirus (RCR) by limiting the packaging constructs to only the necessary coding regions of the viral structural genes and separating them onto different plasmids. This design requires at least three recombination events to produce RCR, thereby enhancing biosafety. Plat-E has proven useful in gene transfer applications, including efficient transduction of primary cells such as T cells and mast cells. Its performance and long-term stability make it a reliable platform for retroviral vector production in both basic research and preclinical gene therapy development.

Organism Human

Tissue Fetal kidney

Synonyms Platinum-E

Características

Age Fetus

Gender Female

Growth properties Adherent

Dados regulatórios

Citation PLAT-E (Cytion catalog number 305855)

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Biosafety level 1**NCBI_TaxID** 9606**CellosaurusAccession** CVCL_B488**GMO Status** GMO-S1: This retroviral packaging cell line (PLAT-E) contains constructs encoding gag-pol and env under EF1 α promoter control, supporting production of ecotropic retroviral particles. The modifications are stably present in HEK293T-derived cells. This classification applies only within Germany and may differ elsewhere.**Dados biomoleculares****Mutational profile****Manuseio****Culture Medium** RPMI 1640, w: 2.0 mM stable Glutamine, w: 2.0 g/L NaHCO₃ (Cytion article number 820700a)**Supplements** Supplement the medium with 10% FBS**Dissociation Reagent** Accutase**Seeding density** 1 to 4 x 10⁴ cells/cm²**Fluid renewal** 2 to 3 times per week**Freeze medium** As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

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Thawing and Culturing Cells

1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
2. Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
3. For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
6. Centrifuge the mixture at $300 \times g$ for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

Incubation Atmosphere

37°C , 5% CO_2 , humidified atmosphere.

Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately -78°C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

Storage Conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about -150 to -196°C . Storage at -80°C is acceptable only as a short interim step before transfer to liquid nitrogen.

Controle de Qualidade e Análise Molecular

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Sterility

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.

To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.