

PK-15 Cells | 607426

Renseignements généraux

Description

The PK(15) cell line, derived from PK-2A, a cell line established in 1955 from the kidney of an adult pig, is infected with the porcine type-C oncovirus (formerly known as porcine endogenous retrovirus, PERV), which is classified as a risk group 2 agent. The host cell genome contains 62 copies of the *pol* gene, which codes for reverse transcriptase and other proteins.

Initially, the virus particles produced by the PK(15) cell line were described as defective and non-infectious for a variety of mammalian cell lines, including a human cell line, leading to its classification as a risk group 1 cell line. However, subsequent studies demonstrated that human 293 cells could be productively infected by the cell-free supernatant of PK(15) cells. This finding resulted in the reclassification of the PK(15) cell line by the German Central Commission for Biological Safety (ZKBS) in November 2018.

PCR analyses revealed that the transmitted viruses belonged to the polytropic subtypes PERV-A and PERV-B. Additionally, it was observed that the virus particles produced by the 293 cells were resistant to inactivation by the human complement system.

In addition to its virological significance, the PK(15) cell line also serves as a suitable host for transfection applications. Due to its adherent growth properties, it is highly valuable in various research and experimental settings.

Organism Pig

Tissue Kidney

Synonyms PK(15), PK (15), PK 15, PK15, Porcine Kidney-15

Caractéristiques

Breed/Subspecies Hampshire

Age Adult

Gender Male

Morphology Epithelial-like

Growth properties Monolayer, adherent

Données réglementaires

Citation PK-15 (Cytion catalog number 607426)

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Biosafety level 1**NCBI_TaxID** 9823**CellosaurusAccession** CVCL_2160**Données biomoléculaires****Viruses** PCV1 (Porcine circovirus 1) positive, PCV2 negative, PCV3 negative**Virus susceptibility** Hog cholera, African swine fever, vesicular exanthema of swine, foot and mouth disease (FMDV), vesicular stomatitis (Indiana), vaccinia, reovirus 2, 3, adenovirus 4, 5, coxsackievirus B2, B3, B4, B5, B6**Virus resistance** Poliovirus 2**Reverse transcriptase** Positive**Manipulation****Culture Medium** EMEM (MEM Eagle), w: 2 mM L-Glutamine, w: 2.2 g/L NaHCO₃, w: EBSS (Cytion article number 820100a)**Supplements** Supplement the medium with 10% FBS and 1% NEAA**Dissociation Reagent** Accutase**Subculturing** Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.**Seeding density** 2×10^4 cells/cm²**Fluid renewal** 2 to 3 times per week**Post-Thaw Recovery** Allow the cells to recover from the freezing process for at least 24 to 48 hours.

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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.

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.

Contrôle de la qualité et analyse moléculaire

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.