

**LLC-PK1 Cells | 607264**

**General information**

**Description**

LLC-PK1 cells are a well-established and widely used cell line in biomedical research. These cells were derived from a healthy male pig's kidney, exhibiting typical epithelial morphology. The LLC-PK1 line is polarized and contains tight junctions, making it an ideal model for epithelial tissue.

One of the critical features of LLC-PK1 cells is their ability to produce plasminogen activator, a substance that stimulates fibrinolysis. This property has made LLC-PK1 cells particularly valuable in thrombosis research.

In recent years, plasminogen activator has been included in drugs used in thrombosis therapies since it facilitates the dissolution of small blood clots. In addition to producing plasminogen activators, LLC-PK1 cells produce large amounts of cytokeratin. This characteristic has made them popular for various pharmacologic and metabolic research investigations.

The LLC-PK1 line has been used in drug metabolism, transport, toxicity, and interaction studies. LLC-PK1 cells are also frequently used in permeability assays. The mechanism of uracil transport differs depending on cell lines, with a Na<sup>+</sup>-independent system on the basolateral membrane in Caco-2 cells and both Na<sup>+</sup>-dependent and Na<sup>+</sup>-independent systems on the apical membrane in LLC-PK1 cells.

Compared to other cell lines, LLC-PK1 cells share many characteristics of proximal tubular cells *in vivo*, including apical membrane microvilli, high activities of apical membrane enzymes, and expression of parathyroid hormone receptors and sodium-dependent glucose transporters. This makes LLC-PK1 cells a valuable tool in renal toxicology studies. Another cell line commonly used in renal toxicology studies is the MDCK cell line. Like LLC-PK1 cells, MDCK cells are epithelial but have characteristics more typical of distal tubular cells.

They express vasopressin, oxytocin, and prostaglandin receptors, which, when stimulated, activate adenylate cyclase. LLC-PK1 and MDCK cell lines proliferate rapidly and can be passaged easily for many generations in monolayer cultures. LLC-PK1 cells are also capable of forming 'domes', fluid-filled blisters resulting from water and solute transport, tight junctions, and adhesion of the cells to the substratum.

In conclusion, the LLC-PK1 cell line is a versatile and valuable tool for biomedical research. It has been widely used in various studies on drug metabolism, drug transport, drug toxicity, drug-drug interactions, renal toxicology, and permeability assays. With its well-established epithelial morphology and plasminogen activator and cytokeratin production, LLC-PK1 cells are an ideal model for epithelial tissue.

**Organism** Sus Scrofa

**Tissue** Kidney

**Applications** Drug metabolism, permeability assays, toxicity, and interaction studies.

**Synonyms** LLC-PK(1), LLC-PK-1, LLC PK-1, Llc-PK1, LLC PK1, LLCPK1, Lilly Laboratories Cell-Porcine Kidney 1

**Characteristics**

**Breed/Subspecies** Hampshire

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**Age** 3-4 weeks

**Gender** Male

**Morphology** Epithelial-like

**Growth properties** Adherent

### Regulatory Data

**Citation** LLC-PK1 (Cytion catalog number 607264)

**Biosafety level** The cell line contains Porcine type-C oncovirus (PCOV) sequences and transcripts. The infection mode is undetermined, and viral secretion cannot be excluded. In Germany, these viruses are classified as BSL 1 for humans and BSL 2 for animals (TRBA 462). However, the German Central Committee on Biological Safety (ZKBS) classifies these viruses and infected cell lines as BSL 2 for genetic modification applications.

**NCBI\_TaxID** 9823

**CellosaurusAccession** CVCL\_0391

### Biomolecular Data

**Viruses** Contains Porcine type-C oncovirus (PCOV) sequences and transcripts. Virus expression cannot be excluded.

**Products** Plasminogen activator

### Handling

**Culture Medium** Medium 199, w: 2.7 mM stable Glutamine, w: 2.2 g/L NaHCO<sub>3</sub>, w: EBSS (Cytion article number 820101a)

**Supplements** Supplement the medium with 3% FBS

**Dissociation Reagent** Accutase

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**Subculturing** Gather the suspension cells in a 15 ml tube and gently wash the adherent cells with PBS lacking calcium and magnesium (use 3-5 ml for T25 flasks and 5-10 ml for T75 flasks). Apply Accutase (1-2 ml for T25 flasks, 2.5 ml for T75 flasks) ensuring full coverage of the cell layer. Allow the cells to incubate at room temperature for 10 minutes. Following incubation, combine and centrifuge both the suspension and adherent cells. After centrifugation, carefully resuspend the cell pellet and transfer the cell suspension into new flasks containing fresh medium.

**Seeding density** 1 to  $3 \times 10^6$  cells/cm<sup>2</sup>

**Fluid renewal** Every 3 days

**Post-Thaw Recovery** After thawing, plate the cells at  $5 \times 10^4$  cells/cm<sup>2</sup> and allow the cells to recover from the freezing process and to adhere for at least 24 hours.

**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  $-150^{\circ}\text{C}$  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  $37^{\circ}\text{C}$  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

$37^{\circ}\text{C}$ , 5%  $\text{CO}_2$ , humidified atmosphere.

### Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately  $-78^{\circ}\text{C}$  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  $-196^{\circ}\text{C}$ . Storage at  $-80^{\circ}\text{C}$  is acceptable only as a short interim step before transfer to liquid nitrogen.

## Quality Control & Molecular Analysis

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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.