

Code:	0355
Cell Line:	PK-15
Species:	Sus scrofa
	Vulgar Name: Pig
Tissue:	Kidney
Morphology:	Epithelial
Growth Properties:	Adherent
Age Ethnicity:	Adult
Applications:	This cell line is a suitable transfection host.
Virus Susceptibility:	Classical swine fever virus , Classical swine fever virus African swine fever virus Vesicular stomatitis, Glasgow (Indiana) Vesicular stomatitis, Orsay (Indiana) Vaccinia virus Human adenovirus 4 Human adenovirus 5 Human Coxsackievirus B 2 Human Coxsackievirus B3 Human Coxsackievirus B 4 Human Coxsackievirus B 5 Human Coxsackievirus B 6
Virus Resistance:	poliovirus 2
Products:	Plasminogen activator; keratin
Biosafety:	1
Additional info:	The presence of a porcine papovavirus in PK(15) cells has been reported in cells obtained from multiple sources. The cell line harbors an endogenous C-type retrovirus. The cells are positive for porcine circovirus (PCV) antigens. The cells are positive for keratin by immunoperoxidase staining.
Culture Medium:	Dulbecco's Modified Eagle's Medium (DMEM) with 1% non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate and 10% FETAL BOVINE SERUM.
Subculturing:	Volumes used in this protocol are for 75 cm ² flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Remove and discard culture medium.

Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum that contains trypsin inhibitor. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. Add appropriate aliquots of the cell suspension to new culture vessels. Incubate cultures at 37°C. NOTE: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 6th edition, published by Alan R. Liss, N.Y., 2010.

Medium Renewal: 2 to 3 times per week

Subcultivation ratio: 1:2 to 1:4 is recommended

Culture Conditions: Atmosphere: air, 95%; carbon dioxide (CO₂), 5% Temperature: 37°C

Cryopreservation: 95% FBS + 5% DMSO (Dimethyl sulfoxide)

Thawing Frozen Cells: SAFETY PRECAUTION: Is highly recommend that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the Oring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. For cells that are sensitive to DMSO is recommended that the cryoprotective agent be removed immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125 x g for 5 to 7 minutes.
4. Discard the supernatant and Resuspend cell pellet with the

recommended complete medium (see the specific batch information for the culture recommended dilution ratio). 5. Incubate the culture in a appropriate atmosphere and temperature (see "Culture Conditions" for this cell line). NOTE: It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).

References:

Dulac GC, Afshar A. Porcine circovirus antigens in PK-15 cell line (ATCC CCL-33) and evidence of antibodies to circovirus in Canadian pigs. *Can. J. Vet. Res.* 53: 431-433, 1989. PubMed: 2686830 Pirtle EC, Woods LK. Cytogenetic alterations in swine kidney cells persistently infected with hog cholera virus and propagated with and without antiserum in the medium. *Am. J. Vet. Res.* 29: 153-164, 1968. PubMed: 4965860 Armstrong JA, et al. C-type virus particles in pig kidney cell lines. *J. Gen. Virol.* 10: 195-198, 1971. PubMed: 4324256 Newman JT, Smith KO. Characteristics of a swine papovavirus. *Infect. Immun.* 5: 961-967, 1972. PubMed: 4344097 Tumilowicz JJ, et al. Concurrent replication of a papovavirus and a C-type virus in the CCL 33 porcine cell line. *In Vitro* 15: 922-928, 1979. PubMed: 232060 Todaro GJ, et al. Characterization of a type C virus released from the porcine cell line PK(15). *Virology* 58: 65-74, 1974. PubMed: 4132403

Depositors:

Ana Jacqueline Bastos, LANAGRO

ATCC:

CCL-33