BCRJ Code:	0355
Cell Line:	PK-15
Species:	Sus scrofa
Vulgar Name:	Pig
Tissue:	Kidney
Morphology:	Epithelial
Growth Properties:	Adherent
Age/Ethinicity:	Adult /
Applications:	This cell line is a suitable transfection host.
Virus Succeptility::	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
Addtional 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 2 mM L-glutamine and 10% of fetal bovine serum.

Subculturing:	Volumes used in this protocol are for 75 cm2 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.
Subculturing Medium Renewal:	2 to 3 times per week
Subculturing 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: It is strongly recommended to always wear protective gloves, clothing, and a full-face mask when handling frozen vials. Some vials may leak when submerged in liquid nitrogen, allowing nitrogen to slowly enter the vial. Upon thawing, the conversion of liquid nitrogen back to its gas phase may cause the vial to explode or eject its cap with significant force, creating flying debris.
1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as its contents are thawed and decontaminate it by dipping in or spraying with 70% ethanol. From this point, all operations must be performed under strict aseptic conditions.
3. For cells sensitive to DMSO, it is recommended to remove the cryoprotective agent immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge at approximately 125 × g for 5 to 7 minutes.
4. Discard the supernatant and resuspend the cell pellet in the recommended complete medium (see specific batch information for the appropriate dilution ratio).
5. Incubate the culture under appropriate atmospheric and temperature conditions (see "Culture Conditions" for this cell line).

NOTE: It is important to avoid excessive alkalinity of the medium during cell recovery. To minimize this risk, it is recommended to place the culture vessel containing the growth medium in the incubator for at least 15 minutes before adding the vial contents. This allows the medium to stabilize at 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

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