

Data Sheet

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BCRJ Code:	0146
Cell Line:	LLC-MK2 Original
Species:	Macaca mulatta
Vulgar Name:	Monkey; Rhesus Monkey
Tissue:	Kidney
Morphology:	Epithelial
Disease:	Normal
Growth Properties:	Adherent
Age/Ethnicity:	Adult /
Virus Susceptibility::	Bovine viral diarrhea virus 1 , Bovine viral diarrhea virus 1 Human poliovirus 1 Human Cocksackievirus B 4
Biosafety:	1
Additional Info:	The serum used in the medium should be pre-tested for good quality. Some lots of horse serum do support satisfactory growth. Adaptation to other types of sera generally results in slow growth with a prolonged lag in growth.
Culture Medium:	Dulbecco's Modified Eagle's Medium (DMEM) modified to contain 2 mM L-glutamine, 4500 mg/L glucose and fetal bovine serum to a final concentration of 10%.

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Subculturing:

Volumes used in this protocol are for 75 cm² flasks; 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 which 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:

Every 2 to 3 days

Subculturing Subcultivation Ratio:

1:4 to 1:6

Culture Conditions:

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

Cryopreservation:

95% FBS + 5% DMSO (Dimethyl sulfoxide)

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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).

American Association of Anatomists. Sixty-ninth annual session; Marquette University School of Medicine, Milwaukee, Wisconsin. Anat. Rec. 124: 490, 1956. Hull RN, et al. Growth characteristics of monkey kidney cell strains LLC-MK1, LLC-MK2, and LLC-MK2(NCTC-3196) and their utility in virus research. J. Exp. Med. 115: 903-918, 1962. PubMed: 14449901 Hull RN, Tritch OJ. Characterization of cell strains by viral susceptibility. Natl. Cancer Inst. Monogr. 7: 161-172, 1962. PubMed: 14449902

Thawing Frozen Cells:

References:

Depositors:

Wanderley de Souza - Universidade Federal do Rio de Janeiro.

Cellosaurus:

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