

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 Coxsackievirus 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 4 mM L- glutamine, 4500 mg/L glucose, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate and fetal bovine serum to a final concentration of 5%.

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.

Medium Renewal: Every 2 to 3 days.

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)

**Thawing Frozen
Cells:**

SAFETY PRECAUTION: It is highly recommended 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 submerged 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 it 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 an 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:

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

Depositors:

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ATCC:

CCL-7