

Code:	0009
Cell Line:	293 [HEK-293]
Species:	Homo sapiens Vulgar Name: Human
Tissue:	Embryonic Kidney
Morphology:	Epithelial
Growth Properties:	Adherent
Age Ethnicity:	fetus
Derivation:	Established from a human primary embryonal kidney transformed by adenovirus type 5; cell line also known as HEK-293 (human embryonic kidney-293)
Applications:	efficacy testing transfection host viruscide testing
Virus Susceptibility:	ADENOVIRUS: PARTICULARLY SENSITIVE TO HUMAN ADENOV
Tumor Formation:	YES
Biosafety:	2
Additional info:	Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome [RF32764], it is now clear that only left end sequences are present. The cells express an unusual cell surface receptor for vitronectin composed of the integrin beta-1 subunit and the vitronectin receptor alpha-v subunit. The Ad5 insert was cloned and sequenced, and it was determined that a colinear segment from nts 1 to 4344 is integrated into chromosome 19 (19q13.2).
Culture Medium:	Dulbecco's modified Eagle's medium. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. Non-essential amino acids, 2 mM L-glutamine, 1 mM sodium pyruvate, and 1500 mg/L sodium bicarbonate.

Subculturing:

1- Remove and discard culture medium. 2- Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3- 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. 4- Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. 5- Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 2×10^3 to 6×10^3 viable cells/cm² is recommended. 6- Incubate cultures at 37°C. 7- Subculture when cell concentration is between 6 and 7×10^4 cells/cm². PLEASE NOTE: Hek293 cells detach at room temperature; may take up to seven days to reattach. 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: 1:6 to 1:10 weekly

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 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).

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Depositors: Didier Petit, Inserm U233, Through Dr. Jose Paulo Leite, Instituto Oswaldo Cruz, Rj

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