

Data Sheet

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BCRJ Code:	0009
Cell Line:	293 [HEK-293]
Species:	Homo sapiens
Vulgar Name:	Human
Tissue:	Embryonic Kidney
Cell Type:	Epithelial
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 with fetal bovine serum to a final concentration of 10% and 1% Non-essential amino acids.
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 x 10 ³ to 6 x 10 ³ viable cells/cm ² is recommended. 6- Incubate cultures at 37°C. 7- Subculture when cell concentration is between 6 and 7 x 10 ⁴ cells/cm ² . 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.
Subculturing Medium Renewal:	Every 2 to 3 days

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**Subculturing
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 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).

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