

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



**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 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 vial 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).

Xie QW, et al. Complementation analysis of mutants of nitric oxide synthase reveals that the active site requires two hemes. Proc. Natl. Acad. Sci. USA 93: 4891-4896, 1996. PubMed: 8643499 Da Costa LT, et al. Converting cancer genes into killer genes. Proc. Natl. Acad. Sci. USA 93: 4192-4196, 1996. PubMed: 8633039 Graham FL, et al. Characteristics of a human cell line transformed by DNA from human adenovirus type 5. J. Gen. Virol. 36: 59-72, 1977. PubMed: 886304 Graham FL, et al. Defective transforming capacity of adenovirus type 5 host-range mutants. Virology 86: 10-21, 1978. PubMed: 664220 Harrison T, et al. Host-range mutants of adenovirus type 5 defective for growth in HeLa cells. Virology 77: 319-329, 1977. PubMed: 841862 Bodary SC, McLean JW. The integrin beta 1 subunit associates with the vitronectin receptor alpha v subunit to form a novel vitronectin receptor in a human embryonic kidney cell line. J. Biol. Chem. 265: 5938-5941, 1990. PubMed: 1690718 Goodrum FD, Ornelles DA. The early region 1B 55-kilodalton oncoprotein of adenovirus relieves growth restrictions imposed on viral replication by the cell cycle. J. Virol. 71: 548-561, 1997. PubMed: 8985383 Loffler S, et al. CD9, a tetraspan transmembrane protein, renders cells susceptible to canine distemper virus. J. Virol. 71: 42-49, 1997. PubMed: 8985321 Hu SX, et al. Development of an adenovirus vector with tetracycline-regulatable human tumor necrosis factor alpha gene expression. Cancer Res. 57: 3339-3343, 1997. PubMed: 9269991 Kolanus W, et al. alphaLbeta2 integrin/LFA-1 binding to ICAM-1 induced by cytohesin-1 a cytoplasmic regulatory molecule. Cell 86: 233-242, 1996. PubMed: 8706128 Stauderman KA, et al. Characterization of human recombinant neuronal nicotinic acetylcholine receptor subunit combinations alpha 2 beta 4, alpha 3 beta 4 and alpha 4 beta 4 stably expressed in HEK293 cells. J. Pharmacol. Exp. Ther. 284: 777-789, 1998. PubMed: 9454827 Bartz SR, et al. Human immunodeficiency virus type 1 cell cycle control: Vpr is cytostatic and mediates G2 accumulation by a mechanism which differs from DNA damage checkpoint control. J. Virol. 70: 2324-2331, 1996. PubMed: 8642659 Sandri-Goldin RM, Hibbard MK. The herpes simplex virus type 1 regulatory protein ICP27 coimmunoprecipitates with anti-sm antiserum, and the C terminus appears to be required for this interaction. J. Virol. 70: 108-118, 1996. PubMed: 8523514 Ansieau S, et al. Tumor necrosis factor receptor-associated factor (TRAF)-1, TRAF-2, and TRAF-3 interact in vivo with the CD30 cytoplasmic domain; TRAF-2 mediates CD30-induced nuclear factor kappa B activation. Proc. Natl. Acad. Sci. USA 93: 14053-14058, 1996. PubMed: 8943059 Zhang J, et al. Dynamin and beta-arrestin reveal distinct mechanisms for G protein-coupled receptor internalization. J. Biol. Chem. 271: 18302-18305, 1996. PubMed: 8702465 Oppermann M, et al. Monoclonal antibodies reveal receptor specificity among G-protein-coupled receptor kinases. Proc. Natl. Acad. Sci. USA 93: 7649-7654, 1996. PubMed: 8755530 Xia Y, et al. Nitric oxide synthase generates superoxide and nitric oxide in arginine-depleted cells leading to peroxynitrite-mediated cellular injury. Proc. Natl. Acad. Sci. USA 93: 6770-6774, 1996. PubMed: 8692893 Zhu X, et al. Cell cycle-dependent modulation of telomerase activity in tumor cells. Proc. Natl. Acad. Sci. USA 93: 6091-6095, 1996. PubMed: 8650224 Uebele VN, et al. Functional differences in Kv1.5 currents expressed in mammalian cell lines are due to the presence of endogenous Kvbeta2.1 subunits. J. Biol. Chem. 271: 2406-2412, 1996. PubMed: 8576199 Abell A, et al. Deletions of portions of the extracellular loops of the lutropin/choriogonadotropin receptor decrease the binding affinity for ovine luteinizing hormone, but not human choriogonadotropin, by preventing the formation of mature cell surface receptor. J. Biol. Chem. 271: 4518-4527, 1996. PubMed: 8626807 Tiberi M, et al. Differential regulation of dopamine D1A receptor responsiveness by various G protein-coupled receptor kinases. J. Biol. Chem. 271: 3771-3778, 1996. PubMed: 8631993 Shahrestanifar M, et al. Studies on inhibition of mu and delta opioid receptor binding by dithiothreitol and N-ethylmaleimide. His223 is critical for mu opioid receptor binding and inactivation by N-ethylmaleimide. J. Biol. Chem. 271: 5505-5512, 1996. PubMed: 8621408 Boring L, et al. Molecular cloning and functional expression of murine JE (monocyte chemoattractant protein 1) and murine macrophage inflammatory protein 1alpha receptors. J. Biol. Chem. 271: 7551-7558, 1996. PubMed: 8631787 Noonberg SB, et al. Evidence of post-transcriptional regulation of U6 small nuclear RNA. J. Biol. Chem. 271: 10477-10481, 1996. PubMed: 8631843 Fox JC, Shanley JR. Antisense inhibition of basic fibroblast growth factor induces apoptosis in vascular smooth muscle cells. J. Biol. Chem. 271: 12578-12584, 1996. PubMed: 8647868 Lee MJ, et al. The inducible G protein-coupled receptor edg-1 signals via the Gi/mitogen-activated protein kinase pathway. J. Biol. Chem. 271: 11272-11279, 1996. PubMed: 8626678 Marchand P, et al. Cysteine mutations in the MAM domain result in monomeric meprin and alter stability and activity of the proteinase. J. Biol. Chem. 271: 24236-24241, 1996. PubMed: 8798668 Arai H, Charo IF. Differential regulation of G-protein-mediated signaling by chemokine receptors. J. Biol. Chem. 271: 21814-21819, 1996. PubMed: 8702980 Huang Q, et al. Substrate recognition by tissue factor-factor VIIa. J. Biol. Chem. 271: 21752-21757, 1996. PubMed: 8702971 Monteclaro FS, Charo IF. The amino-terminal extracellular domain of the MCP-1 receptor, but not the RANTES/MIP-1alpha receptor, confers chemokine selectivity. J. Biol. Chem. 271: 19084-19092, 1996. PubMed: 8702581 Keith DE, et al. Morphine activates opioid receptors without causing their rapid internalization. J. Biol. Chem. 271: 19021-19024, 1996. PubMed: 8702570 Louis N, et al. Cloning and sequencing of the cellular-viral junctions from the human adenovirus type 5 transformed 293 cell line. Virology 233: 423-429, 1997. PubMed: 9217065 Shaw G, et al. Preferential transformation of human neuronal cells by human adenoviruses and the origin of HEK 293 cells. FASEB J. 16: 869-871, 2002. PubMed: 11967234 Standard Test Method for Determining the Virus-Eliminating Effectiveness of Liquid Hygienic Handwash and Handrub Agents Using the Fingerpads of Adult Volunteers. West Conshohocken, PA:ASTM International;ASTM Standard Test Method E 1838-02. Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporocidal Activities of Liquid Chemical Germicides. West Conshohocken, PA:ASTM International;ASTM Standard Test Method E 2197-02.

References:

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ATCC: CRL-1573

