

HEK293 Cells | 300192**General information****Description**

The HEK293 cell line, an immortalized epithelial cell line derived from human embryonic kidney cells in the 1970s by Alex van der Eb at the University of Utrecht, has become a pivotal experimental model in molecular biology and biotechnological applications due to its remarkable versatility and ease of genetic manipulation.

The transformation of the HEK293 cell line involved the integration of a specific segment from Adenovirus 5 DNA, embedding the adenoviral E1A and E1B genes within the cellular genome. The adenoviral DNA modification enabled the cell lines' ability to uptake foreign DNA efficiently, a feature known as high transfection efficiency. The integration of viral DNA into the HEK293 cell genome resulted in cellular immortalization and significantly enhanced the utility of these cells in biotechnological applications by facilitating the stable incorporation and expression of exogenous DNA, a process termed stable transfection. This capability allows for the persistent presence and function of foreign genes within the cells, making HEK293 an invaluable tool for genetic studies and biotechnology.

As a result, HEK293 cells have become a fundamental resource in biotechnology for the production of recombinant proteins, including vital therapeutic proteins, and serving as robust host cells for the generation of viral vectors, particularly adenoviral and lentiviral vectors. HEK 293 cells are pivotal in the pharmaceutical industry for high-throughput screening assays, the manufacture of gene therapies targeting specific genes related to single gene disorders, and adenoviral infection studies.

In industrial biotechnology, the utility of the human cell line HEK293 extends to recombinant enzyme production, viral vector production, such as adenoviral vectors, protein production and the development of biosensors. Toxicology research benefits from the application of the HEK cell line in assessing the impacts of chemicals on cell biology, including the effects on typical kidney cells and the potential for gene therapies. The ability of the immortal cell line HEK293 to efficiently produce native proteins highlights their essential role in medical research, including cancer research and exploring the foundations of gene therapy.

HEK293 cells offer a unique platform for studying cell biology and proteins of interest, surpassing other cell lines in versatility and utility in both research and industrial applications. In comparison, HEK293T cells, a variant of HEK293, are modified to enhance transfection efficiency, HEK293F cells are adapted for suspension culture to facilitate large-scale protein production, and other mammalian cell lines such as Vero cells, derived from monkey kidney tissue, are primarily utilized in vaccine development and viral studies.

Organism	Human
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Tissue	Kidney
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Applications	Transfection host
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Synonyms	Hek293, HEK-293, HEK/293, HEK 293, HEK,293, 293, 293 HEK, 293 Ad5, Human Embryonic Kidney 293
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Characteristics

Age	Fetus
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Gender	Female
Morphology	Epithelial-like
Growth properties	Monolayer, adherent

Regulatory Data

Citation	HEK293 (Cytion catalog number 300192)
Biosafety level	1
NCBI_TaxID	9606
CellosaurusAccession	CVCL_0045
GMO Status	GMO-S1: This HEK293 embryonic kidney-derived cell line contains adenovirus-5 E1A/E1B sequences due to transformation but does not release infectious virus, enabling high proliferative capacity. The modification is stably present in embryonal kidney cells. This classification applies only within Germany and may differ elsewhere.

Biomolecular Data

Receptors expressed	Vitronectin
Protein expression	CEA negative, p53 positive
Tumorigenic	In nude mice
Virus susceptibility	Transformed with adenovirus 5 DNA adenovirus 5 DNA
Ploidy status	30% of HEK293 cells have hypotriploid karyotypes with 64 modal chromosomes. Higher ploidies were found in 4.2% of cells.

Handling

Culture Medium	EMEM (MEM Eagle), w: 2 mM L-Glutamine, w: 2.2 g/L NaHCO ₃ , w: EBSS (Cytion article number 820100a)
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Supplements Supplement the medium with 10% FBS and 1% NEAA

Dissociation Reagent Accutase

Doubling time 30 hours

Subculturing Remove the old medium from the adherent cells and wash them with PBS that lacks calcium and magnesium. For T25 flasks, use 3-5 ml of PBS, and for T75 flasks, use 5-10 ml. Then, cover the cells completely with Accutase, using 1-2 ml for T25 flasks and 2.5 ml for T75 flasks. Let the cells incubate at room temperature for 8-10 minutes to detach them. After incubation, gently mix the cells with 10 ml of medium to resuspend them, then centrifuge at 300xg for 3 minutes. Discard the supernatant, resuspend the cells in fresh medium, and transfer them into new flasks that already contain fresh medium.

Seeding density 1×10^4 cells/cm² will yield in a confluent layer in about 4 days.

Fluid renewal 2 times per week

Post-Thaw Recovery After thawing, plate the cells at 5×10^4 cells/cm² and allow the cells to recover from the freezing process and to adhere for at least 24 hours.

Freeze medium As a cryopreservation medium, we use complete growth medium (including FBS) + 10% DMSO for adequate post-thaw viability, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

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Thawing and Culturing Cells

1. Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
2. Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
3. For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
4. Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
5. Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
6. Centrifuge the mixture at $300 \times g$ for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
7. Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
8. Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

Incubation Atmosphere

37°C , 5% CO_2 , humidified atmosphere.

Shipping Conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately -78°C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

Storage Conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about -150 to -196°C . Storage at -80°C is acceptable only as a short interim step before transfer to liquid nitrogen.

Quality Control & Molecular Analysis

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Sterility

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.

To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.