

HEK293 EBNA Cells | 300264**General information****Description**

The HEK293 EBNA cell line is a derivative of the original HEK293 line, which itself was derived from human embryonic kidney cells grown in tissue culture. This particular subline was engineered to stably express the Epstein-Barr virus nuclear antigen-1 (EBNA-1). The expression of EBNA-1 allows for the episomal replication of plasmids that carry the EBV origin of replication, making HEK293 EBNA cells particularly valuable for the production of recombinant proteins and for gene expression studies involving episomal vectors.

HEK293 EBNA cells retain many of the characteristics of the parent HEK293 cells, including their adherence to cell culture plastic and their robust growth in standard mammalian cell culture media. The addition of EBNA-1 expands their utility in research and biotechnological applications, as it enhances the cells' ability to propagate plasmids with the EBV origin of plasmid replication. This feature is critical for producing stable, high-yield recombinant proteins, which is essential for both research purposes and industrial-scale production.

Organism Human**Tissue** Embryonic kidney**Synonyms** HEK293-EBNA, 293 c18, 293c18, HEK 293 c18, HEK-293 c18, HEK293-EBNA1, HEK-293-EBNA, HEK 293-EBNA, HEK 293 EBNA, HEK293EBNA, 293 EBNA, 293-EBNA1, 293-EBNA, 293/EBNA, 293EBNA, EBNA-293, EBNA293, HEK293E, HEK/EBNA, HEK-EBNA, HEK.EBNA, 293/EBNA-1, 298E**Characteristics****Age** Fetus**Gender** Female**Morphology** Epithelial**Growth properties** Adherent**Regulatory Data****Citation** HEK293 EBNA (Cytion catalog number 300264)**Biosafety level** 2**NCBI_TaxID** 9606**CellosaurusAccession** CVCL_6974

HEK293 EBNA Cells | 300264**GMO Status**

GMO-S1: This HEK293 EBNA cell line contains EBV nuclear antigen (EBNA) sequences enabling episomal replication of EBV-derived plasmids, without releasing infectious virus particles. The modification is stably present in embryonic kidney-derived cells. This classification applies only within Germany and may differ elsewhere.

Biomolecular Data**Antigen expression**

EBNA1

Viruses

Adenovirus 5 (Transformant), EBV (expresses EBNA1)

Handling**Culture Medium**

DMEM, w: 4.5 g/L Glucose, w: 4 mM L-Glutamine, w: 3.7 g/L NaHCO₃, w: 1.0 mM Sodium pyruvate (Cytion article number 820300a)

Supplements

Supplement the medium with 10% FBS

Dissociation Reagent

Accutase

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.

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.