Description
HEK-293.2sus is an embryonic cell line that was isolated in 2008 from the kidney of a human embryo.

Organism: Homo sapiens, human
Cell Type: embryonic cell
Tissue: kidney; Embryo
Age: fetus
Morphology: rounded
Growth properties: Suspension

Storage Conditions
Product format: Frozen
Storage conditions: Vapor phase of liquid nitrogen

Intended Use
This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 2
ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of Biosafety in Microbiological and Biomedical Laboratories (BMBL), U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization’s policies and procedures as well as any other applicable regulations as enforced by your local
or national agencies.

Cells contain Adenovirus type 5 DNA sequences

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed 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 vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Temperature: 37°C
Atmosphere: 95% Air, 5% CO₂

Handling Procedures

Unpacking and storage instructions:
1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until
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ready for use.

**Complete medium:** The base medium for this cell line is 293 SFM II (Invitrogen, Catalog No. 11686-029). To make the complete growth medium, add the following component to the base medium: 4mM L-glutamine (final conc.)

**Handling Procedure:**

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of 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 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. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete growth medium and spin at approximately 125 x g for 5 to 7 minutes.
4. Resuspend cell pellet with the recommended complete growth medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a 25 cm² or a 75 cm² culture flask. 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 complete 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).
5. Incubate the culture at 37°C in a suitable incubator. A 5% CO₂ in air atmosphere is recommended if using the medium described on this product sheet.

**Subculturing procedure:**

Volumes are given for a 75 cm² flask. Increase or decrease the amount of dissociation medium needed proportionally for culture vessels of other sizes.

1. Cultures can be maintained by addition of fresh medium. Dilute cultures to a cell concentration between 1 x 10⁵ and 3 x 10⁵ cells/mL.
2. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 1 x 10⁵ and 3 x 10⁵ viable cells/mL. Transfer the suspension to a centrifuge tube. Sharply rap the side of the flask against your hand or a
protected surface several times to remove any adherent cells. Resuspend the
dislodged cells in 5 mL medium and triturate with a small bore pipette until
cell clumps are dispersed. Pool resuspended cells into the centrifuge tube.
Centrifuge at 125 xg for 5 to 7 minutes. Remove supernatant and resuspend
the cell pellet with fresh medium.
3. Do not allow the cell concentration to exceed $1 \times 10^6$ cells/mL.

**Medium renewal:** Two to three times weekly

**Reagents for cryopreservation:** Conditioned growth medium (day 3 to 4 cell
conditioned medium collected from HEK-293.2sus cultures during subculture
procedure) supplemented with 10% (v/v) DMSO (ATCC 4-X)

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**Material Citation**

If use of this material results in a scientific publication, please cite the material in the
following manner: HEK-293.2sus (ATCC CRL-1573.3)

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**References**

References and other information relating to this material are available at
www.atcc.org.

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website, and Certificate of Analysis. For living cultures, ATCC lists the media
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While other unspecified media and reagents may also produce satisfactory results, a
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Contact Information
ATCC
10801 University Boulevard
Manassas, VA 20110-2209
USA
US telephone: 800-638-6597
Worldwide telephone: +1-703-365-2700
Email: tech@atcc.org or contact your local distributor