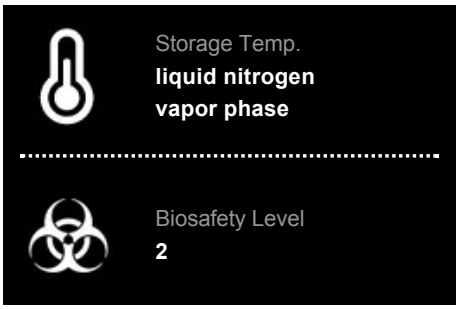
ATCC

Product Sheet

HEK-293.2sus (ATCC® CRL-1573.3™)

Please read this FIRST



Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

Complete Growth 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.)

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: HEK-293.2sus (ATCC® CRL-1573.3™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Organism: *Homo sapiens*, human
Tissue: embryonic kidney
Cell Type: embryonic
Age: fetus
Morphology: rounded
Growth Properties: suspension
DNA Profile:
Amelogenin: X
CSF1PO: 12
D13S317: 12,14
D16S539: 9,13
D5S818: 8
D7S820: 11,12
TH01: 7,9.3
TPOX: 11
vWA: 16,19
Cytogenetic Analysis: The following is based on the parent cell line (ATCC CRL-1573): This is a hypotriploid human cell line. The modal chromosome number was 64, occurring in 30% of cells. The rate of cells with higher ploidies was 4.2 %.
The der(1)t(1;15) (q42;q13), der(19)t(3;19) (q12;q13), der(12)t(8;12) (q22;p13), and four other marker chromosomes were common to most cells. Five other markers occurred in some cells only. The marker der(1) and M8 (or Xq+) were often paired.
There were four copies of N17 and N22. Noticeably in addition to three copies of X chromosomes, there were paired Xq+, and a single Xp+ in most cells.
Note: Cytogenetic instability has been reported in the literature for some cell lines.

Batch-Specific Information

Refer to the Certificate of Analysis for batch-specific test results.

SAFETY PRECAUTION

ATCC highly recommends 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 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 vessel exploding or blowing off its cap with dangerous force creating flying debris.

Unpacking & 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 ready for use.

Handling Procedure for Frozen Cells

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 cm^2 or a 75 cm^2 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% CO_2 in air atmosphere is recommended if

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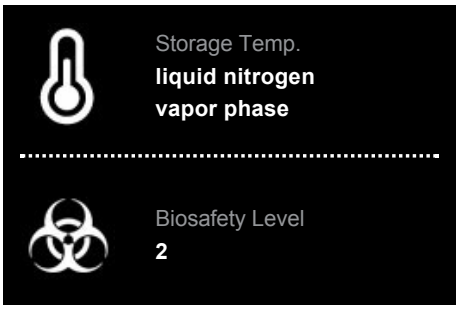

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using the medium described on this product sheet.

Handling Procedure for Flask Cultures

The flask was seeded with cells (see specific batch information), grown, and completely filled with medium at ATCC to prevent loss of cells during shipping.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination. Using an inverted microscope (preferably equipped with phase-contrast optics), carefully check for any evidence of microbial contamination
2. Incubate the flask in an upright position for several hours at 37°C. After the temperature has equilibrated, aseptically remove the entire contents of the flask and centrifuge at 125 xg for 5 to 10 minutes. Remove shipping medium and save for reuse. Resuspend the cell pellet in 10 mL of this medium.
3. From this cell suspension remove a sample for a cell count and viability. Adjust the cell density of the suspension to 3 x 10^5 to 5 x 10^5 viable cells/mL in the shipping medium.
4. Incubate the culture, horizontally, at 37°C in a 5% CO_2 in air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure.

Subculturing Procedure

Volumes are given for a 75 cm^2 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 10^5 and 3 x 10^5 cells/mL.
2. Alternatively, cultures can be established by centrifugation with subsequent resuspension at 1 x 10^5 and 3 x 10^5 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 cell pellet with fresh medium.
3. Do not allow the cell concentration to exceed 1 x 10^6 cells/mL.

Medium renewal: Two to three times weekly

Cryopreservation Medium

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. Cell culture tested DMSO is available as ATCC Catalog No. 4-X.

Comments

ATCC CRL-1573 (HEK293) cells were progressively adapted from an anchorage-dependent growth mode to a suspension mode.
Although an earlier report suggested that the cells contained Adenovirus 5 DNA from both the right and left ends of the viral genome, it is now clear that only left end sequences are present.
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).

References

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

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be

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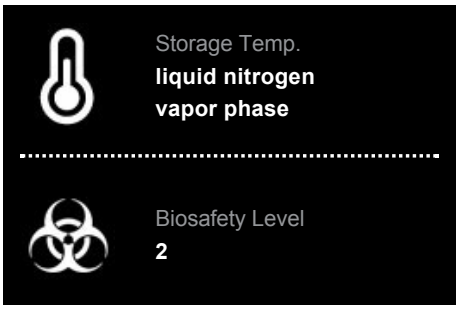

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effective for this product. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.
While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. ATCC does not warrant that such information has been confirmed to be accurate.
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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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