293T/17 SF [HEK 293T/17 SF]

ACS-4500™

Description

HEK293T/17 SF cells are a derivative of the 293T (293tsA1609neo) cell line (ATCC CRL-11268), adapted to serum-free medium and suspension.

Organism: *Homo sapiens*, human
Tissue: kidney
Morphology: Lymphocyte-like; single cells to small aggregates
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

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.

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 ready for use.

Complete medium:

The base medium for this cell line is BalanCD HEK293 (Irvine Scientific cat# 91165). To make the complete medium, add to 475 mL of the base medium:

- 20 mL of 200 mM L-glutamine (ATCC 30-2214) for a final concentration of 8

mM
- 5 mL of ITS (Corning cat# 25-800-CR) for a final concentration of 10 µL/mL

This medium is formulated for use with a 5-8% CO_2 air atmosphere.

Handling Procedure:

To ensure 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 -80°C. Storage at -80°C will result in loss of viability.

1. Rapidly thaw cells by placing the cryovial in a 37°C water bath, swirling gently. Remove the cryovial from the water bath when only a few ice crystals are remaining.
2. Sterilize the cryovial by rinsing with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Using a 1-mL or 5-mL pipette, transfer thawed cells drop-wise into 9 mL pre-warmed complete growth medium in a 15-mL centrifuge tube. Gently pipette the cells up and down several times to mix thoroughly.
4. Centrifuge the cell suspension at 170 × *g* for 5 minutes.
5. Carefully aspirate the supernatant and discard, leaving the cell pellet.
6. Gently resuspend the cell pellet in fresh pre-warmed complete growth medium, and transfer cell suspension into a filtered cap/non-baffled shaker flask. Cells should be seeded at a density of 5 x 10^5 cells/mL.
7. Place the flask in a 37°C shaking incubator (125 to 130 rpm) with 5-8% CO_2.
   Note: Viability after initial thaw is generally lower; however, after 2-3 passages, the cells are fully recovered and reach optimal viability.

Subculturing procedure:

Subculture cells at log phase (when cells are ready for passaging, i.e., every 2-3 days, and are approximately 2 x 10^6 cells/mL). Pre-warm fresh growth medium prior to use. Swirl the flask gently to evenly distribute cells in medium. Remove a small volume of cells from the flask and perform cell count.

1. Seed at 5x10^5 cells/mL for a 2 day subculture and 4x10^5 cells/mL for a 3 day subculture (weekend)
2. To maintain high cell viability, prior to seeding, centrifuge cells for 5min at 170x g
3. Discard spent media and re-suspend cell pellet in pre-warmed fresh complete

growth media

4. Pipette cells gently to break aggregates.

Note: Slight aggregates may be observed, but they are easily dispersed with minimal pipetting and do not impact the performance of the cell line. Alternately, appropriate amount of fresh media maybe added directly into the flask to adjust cell seeding density. However, cell viability might be slightly compromised and decreased by 5%.

Cryopreservation:

Cells should be frozen at a high concentration (e.g., 5-7 x 10^6 cells/mL) and at a low passage number. The cells should be ≥ 85% viable prior to freezing.

1. Prepare 2X freezing medium (Complete Growth Medium supplemented with 15% DMSO) and store at 2°C to 8°C until ready to use.
2. Determine the viable number of cells and percent viability. Calculate the required volume of freezing medium based on the desired viable cell density per vial.
3. Centrifuge the cell suspension at 170 × g for 5 to 10 minutes. Carefully aspirate & discard supernatant.
4. Resuspend the cell pellet in Complete Growth Medium, and then add equal volume of the cold 2X freezing medium (prepared in step 1).
5. Transfer the cell suspension into cryovials (1 mL/vial). Continue to gently mix the cell suspension to avoid cell clumping and to keep the suspension at a homogeneous state.
6. Freeze the cells gradually at a rate of -1°C/min until the temperature reaches -70°C to -80°C. If a controlled rate freezer is not available, an isopropanol freezing container also may be used (e.g., Mr. Frosty). Store cells at -80°C overnight. Follow manufacturer instructions for freezing cells in chambers.
7. The cells should not be left at -80°C for more than 24 to 48 hours. Once at -80°C, frozen cryovials should be transferred to the vapor phase of liquid nitrogen for long-term storage.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: 293T/17 SF [HEK 293T/17 SF] (ATCC ACS-4500)

References

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

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