293SF-3F6

CRL-3537™

Description

293SF-3F6 were adapted to suspension serum-free culture. These cells may be used to produce E1-deleted adenoviral vectors as well as used for transfection for recombinant protein production. This product is an ATCC manufactured and accessioned progeny of CRL-12585 cited in US Pat. No. US 6,210,922.
Organism: *Homo sapiens*, human
Tissue: kidney
Age: embryo
Gender: Female
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.

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% CO_2

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 HyCell TransFx-H. To make the complete medium add these products to the base medium:

- 10 mL L-glutamine (ATCC 30-2214) to a final concentration of 4mM
- 1 mL poloxamer 188 (Fisher Scientific cat # MT13901CI or Corning cat # 13901CI) to a final concentration of 0.2%

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 culture medium and spin at approximately 125 x g for 5 to 7 minutes.
4. Carefully aspirate the supernatant and discard, leaving the cell pellet.
5. Gently resuspend the cell pellet in fresh pre-warmed complete growth medium, and transfer cell suspension into a vented, non-baffled shaker flask. Cells should be seeded at a density of 5 x 10^5 cells/mL.
6. Place the flask in a 37°C shaking incubator (120 to 130 rpm) with 5-8% CO_2.

Subculturing procedure:

Re-seed cells at log phase every 2-3 days when the cell density is between 1.5 x 10^6 and 3.0 x 10^6 viable 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. Cultures can be maintained by addition or replacement of fresh medium. Start cultures at 5.0 X 10^5 cells/mL and maintain between 4 X 10^5 and 3 X 10^6 cells/mL.

1. Seed at 5 x 10^5cells/mL for a 2 day subculture and 4 x 10^5 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.
5. Add the cell suspension to a non-baffled, vented flask.
6. Place the flask in a 37°C shaking incubator (120 to 130 rpm) with 5-8% CO_2.

Medium Renewal: Add fresh medium every 2 to 3 days (depending on cell density). Re-seed cells at log phase every 2-3 days when the cell density is between 1.5 x 10^6 and 3.0 x 10^6 viable cells/mL. Cells should be transferred to a new vessel at each media addition. This is because a ring of adhered cells forms on the shaker flask at the liquid air interface. If media were added to the flask above this line, then adhered cells will dislodge and become part of the cell suspension causing reduced viability.
Reagents for cryopreservation: Complete culture medium + 5% DMSO (ATCC 4-X)
Cryopreservation: Complete culture medium + 5% DMSO (ATCC catalog # 4-X). Cells should be frozen at a high concentration (e.g., 7-8 x 10^6 cells/mL) and at a low passage number. The cells should be ≥ 85% viable prior to freezing.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: 293SF-3F6 (ATCC CRL-3537)

References

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

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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