



Product Sheet

## Serum-Free Cell Freezing Medium (ATCC® 30-2600™)

Please read this FIRST

Storage Temp.  
2°C to 8°C

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Biosafety Level  
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### Description

#### Product Description:

ATCC® Serum-Free Cell Freezing Medium is a sterile, ready-to-use medium suitable for the cryopreservation of adherent and suspension cell cultures. This proprietary, serum-free formulation contains 10% DMSO and methylcellulose.

Cells cryopreserved using Serum-Free Freezing Medium show levels of viability and percent attachment (adherent cells) that are comparable to cells preserved in DMSO and FBS. Serum-Free Cell Freezing Medium can be used for both cells cultured in serum-supplemented growth medium as well as cells grown under serum-free conditions.

**Volume:** 20 mL

### Directions for Use

Cryopreserve cells when cultures are actively growing. If growing adherent cells under serum-free conditions, we recommend the use of ATCC® 30-2101 Trypsin-EDTA Solution (1X) and ATCC® 30-2104 Soybean Trypsin Inhibitor (50X Concentrate) to detach the cells.

1. Harvest the culture to prepare a cell suspension using your standard, cell-specific method.
2. Centrifuge the cells at 125 x g for 5 to 10 minutes.
  - a. Do not over-centrifuge cells as this may cause cell damage.
  - b. After centrifugation, the cells should form a clean loose pellet.
3. Aspirate medium and (neutralized) dissociation solution (used with adherent cells) and resuspend the cell pellet in 2 to 8 mL fresh, pre-warmed, complete growth medium.
4. Count the cells. Centrifuge the cells again at 125 x g for 5 to 10 minutes.
5. Take the Serum-Free Cell Freezing Medium from storage and swirl to mix. Decontaminate the vial by dipping in or spraying with 70% alcohol.
6. Aspirate the medium and suspend the cell pellet in Serum-Free Cell Freezing Medium at a concentration  $3 \times 10^6$  to  $5 \times 10^6$  cells/mL. Aliquot 1 mL of the cell suspension to each labeled cryovial.
7. Freeze the cells gradually at a rate of  $-1^\circ\text{C}/\text{min}$  until the temperature reaches  $-70^\circ\text{C}$  to  $-80^\circ\text{C}$ . The cells should not be left at  $-80^\circ\text{C}$  for more than 24 to 48 hours. Once at  $-80^\circ\text{C}$ , frozen cryovials should be transferred to the vapor phase of liquid nitrogen for long-term storage.

#### Handling Procedure for Frozen Cells and Initiation of Cultures

1. Warm the complete growth medium to  $37^\circ\text{C}$  prior to use with the cells.
2. Thaw a vial of cells cryopreserved in Serum-Free Cell Freezing Medium by gentle agitation in a  $37^\circ\text{C}$  water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 90 seconds).
3. Remove the vial from the water bath before the contents are completely 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.
4. Transfer the vial's contents plus 5 mL of complete growth medium to a 15 mL centrifuge tube. Use an additional 1 mL of medium to rinse the vial and transfer the liquid to the 15 mL tube. Add 4 mL of complete cell growth medium to bring the total volume to 10 mL.
5. Spin the cells at 125 x g for 5 min. Aspirate the supernatant and resuspend the pellet in 2 mL of complete growth medium.
6. Count the cells; adjust the volume so that the cells are plated at the appropriate seeding density.

### ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. 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 strain. 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.

This product is sent with the condition that you are responsible for its safe storage, handling, and use.

American Type Culture Collection  
PO Box 1549  
Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

800.638.6597 or 703.365.2700  
Fax: 703.365.2750  
Email: [Tech@atcc.org](mailto:Tech@atcc.org)

Or contact your local distributor



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ATCC is not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to insure authenticity and reliability of strains on deposit, ATCC is not liable for damages arising from the misidentification or misrepresentation of cultures.

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](http://www.atcc.org)

Additional information on this culture is available on the ATCC web site at [www.atcc.org](http://www.atcc.org).


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American Type Culture Collection  
PO Box 1549  
Manassas, VA 20108 USA  
[www.atcc.org](http://www.atcc.org)

800.638.6597 or 703.365.2700  
Fax: 703.365.2750  
Email: [Tech@atcc.org](mailto:Tech@atcc.org)

*Or contact your local distributor*