

30-2600<sup>TM</sup>

### 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. This product has applications for cyropreservation, cell culture, cell growth, and viability.

Volume: 20 mL

### Storage Conditions

Storage conditions: 2°C to 8°C

#### 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.

### **Biosafety Information**

ATCC determined that a biosafety level is not applicable to this 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 complete your own risk assessment and understand any potential hazards associated with the material per your organization's policies and procedures and any other applicable regulations as enforced by your local or national agencies.

### 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

#### **Cryopreservation of Cells**

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°C/min until the temperature reaches 70°C to -80°C. 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.

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

- 1. Warm the complete growth medium to 37°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°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.

## **Quality Control Specifications**

Mycoplasma contamination: Not detected

#### Notes



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#### Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: Serum-Free Cell Freezing Medium (ATCC 30-2600)

#### References

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

### Warranty

The product is provided 'AS IS' and the viability of ATCC® products is warranted for 30 days from the date of shipment, provided that the customer has stored and handled the product according to the information included on the product information sheet, website, and Certificate of Analysis. For living cultures, ATCC lists the media formulation and reagents that have been found to be effective for the product. While other unspecified media and reagents may also produce satisfactory results, a change in the ATCC and/or depositor-recommended protocols may affect the recovery, growth, and/or function of the product. If an alternative medium formulation or reagent is used, the ATCC warranty for viability is no longer valid. Except as expressly set forth herein, no other warranties of any kind are provided, express or implied, including, but not limited to, any implied warranties of merchantability, fitness for a particular purpose, manufacture according to cGMP

standards, typicality, safety, accuracy, and/or noninfringement.

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#### Revision

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