



Product Sheet

S16 (ATCC® CRL-2941™)

Please read this FIRST



Storage Temp.
liquid nitrogen
vapor phase



Biosafety Level
1

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 ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%.

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: S16 (ATCC® CRL-2941™)

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: *Rattus norvegicus*, rat

Tissue: sciatic nerve

Cell Type: Schwann cell

Age: neonatal

Morphology: epithelial-like

Growth Properties: adherent

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.

Note: The culture flasks should be treated with $0.1\text{mL}/\text{cm}^2$ of flask surface area with $15\mu\text{g}/\text{mL}$ poly-L-lysine (Sigma Cat. #P-9155 or equivalent) for at least 2 hours at 37°C . Remove solution and rinse one time with DPBS and allow flask to air dry uncapped and standing upright in a biological cabinet for about 30 minutes before introducing cells.

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 $125 \times g$ for 5 to 10 minutes.
4. Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio) and dispense into a **poly-L-lysine coated** 25cm^2 or a 75cm^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).

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.

Note: The culture flasks should be treated with $0.1\text{mL}/\text{cm}^2$ of flask surface area with $15\mu\text{g}/\text{mL}$ poly-L-lysine (Sigma Cat. #P-9155 or equivalent) for at least 2 hours at 37°C . Remove solution and rinse one time with DPBS and allow flask to air dry uncapped and standing upright in a biological cabinet for about 30 minutes before introducing cells.



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. Also, check to determine if the majority of cells are still attached to the bottom of the flask; during shipping the cultures are sometimes handled roughly and



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- many of the cells often detach and become suspended in the culture medium (but are still viable).
- If the cells are still attached**, aseptically remove all but 5 to 10 mL of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO₂ in air atmosphere until they are ready to be subcultured.
- If the cells are not attached**, aseptically remove the entire contents of the flask and centrifuge at 125 x g for 5 to 10 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 mL of this medium and add to a **poly-L-lysine coated** 25 cm² flask. Incubate at 37°C in a 5% CO₂ in air atmosphere until cells are ready to be subcultured.



Subculturing Procedure

Volumes used in this protocol are for 75 cm² flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes.

Note:

The culture flasks should be treated with 0.1 mL/cm² of flask surface area with 15ug/mL poly-L-lysine (Sigma Cat. No. P-9155 or equivalent) for at least 2 hours at 37°C. Remove solution and rinse one time with DPBS and allow flask to air dry uncapped and standing upright in a biological cabinet for about 30 minutes before introducing cells.

- Remove and discard culture medium.
- Briefly rinse the cell layer with Ca⁺⁺/Mg⁺⁺ free Dulbecco's phosphate-buffered saline (DPBS) or 0.25% (w/v) Trypsin - 0.53 mM EDTA solution to remove all traces of serum which contains trypsin inhibitor.
- Add 1.0 to 2.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
- Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting.
- Transfer cell suspension to a centrifuge tube and spin at approximately 125 X g for 5 to 10 minutes. Discard supernatant.
- Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 4 X 10³ to 6 X 10⁴ viable cells/cm² is recommended.
- Incubate cultures at 37°C. We recommend that you maintain cultures at a cell concentration between 2 X 10⁴ and 4 X 10⁴ cells/cm².

Subcultivation ratio: A subcultivation ratio of 1:4 to 1:6 twice weekly is recommended.

Medium renewal: Every 2 to 3 days

Interval: Subculture when cell concentration is between 2 x10⁴ and 4x10⁴ cells/cm².



Cryopreservation Medium

Complete growth medium described above supplemented with 10% (v/v) DMSO.

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



Comments

High Myelin-associated glycoprotein (MAG) expression was demonstrated by Radioimmunoassay. The S16 cells divide very rapidly, are rounder than normal Schwann cells and elaborate many processes after reaching high density. The cells express galactocerebroside (GalC), sulfatide and P0 glycoprotein, but little or no myelin basic protein.



References

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



Biosafety Level: 1

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



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Disclaimers

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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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