

50532<sup>™</sup>

### Description

**Strain designation:** SLC 89 **Deposited As:** Oxytricha trifallax

Type strain: No

## Storage Conditions

**Product format:** Frozen

Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for

long-term storage

#### 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<sub>1</sub>

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

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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**

#### **Medium:**

ATCC Medium 802: Sonneborn's Paramecium medium

Instructions for complete medium: ATCC Medium 802 inoculated with *Klebsiella pneumoniae* subsp. *pneumoniae* (ATCC® 700831™) or *Enterobacter aerogenes* (ATCC® 13048™). Culture also contains unidentified microalgae as food source.

**Temperature:** 20-25°C **Culture system:** Xenic

Incubation: With Enterobacter aerogenes ATCC 13048 and unidentified microalgae as

food sources

## Handling Procedures

#### **Handling of Live Culture**

This strain is routinely shipped as a growing culture in a glass 16 x 125 mm screw-

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capped test tube. The volume of the cell suspension is approximately 5 mL. When the culture arrives remove it promptly from the shipping container. **Do not store the culture at refrigeration temperatures before handling.** To assure viability, immediately loosen the test tube cap and incubate upright at 25°C for at least one hour before observing the culture. There should be numerous active trophozoites in suspension. If the numbers are low or cysts are present, the culture may have been exposed to temperature extremes in transit. Regardless of the state of the culture, aseptically transfer a 0.5-1.0 mL aliquot to a 16 x 125 mm screw-capped test tube containing 5 mL of bacterized ATCC Medium 802. Incubate the parent and daughter cultures upright with the caps on loosely at 25°C.

**Culture maintenance:** Subculture every week to a fresh tube of bacterized medium in the following manner:

- 1. Transfer 0.5-1.0 mL from a growing culture to 5.0 mL of bacterized ATCC medium 802.
- 2. Add 1.0 mL of bacterized ATCC medium 802 twice weekly. When the tube is filled to within one inch of the top, aspirate from the bottom of the tube and reduce the volume to 5.0 mL.
- 3. Incubate upright at 25°C with the cap on loosely.

#### Reagents for cryopreservation: Cryoprotective Solution

DMSO. 2.0 mL

Fresh growth medium w/o bacteria, 8.0 mL

#### **Cryopreservation:**

#### **Harvest and Preservation**

- 1. Mix the components in the order listed. When the medium is added to the DMSO the solution will warm up due to chemical heat.
- 2. Harvest cells from a culture that is at or near peak density by centrifugation at  $125 \times g$  for 5 min.
- 3. Adjust the concentration of cells at least 1 x  $10^5$ /mL in fresh medium.
- 4. Mix the cell preparation and the cryoprotective solution in equal portions.
- 5. Dispense in 0.5 mL aliquots into 1.0 2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation).
- 6. Place vials in a controlled rate freezing unit. From room temperature cool at 1°C/min to -40°C. If freezing unit can compensate for the heat of fusion, maintain rate at -1°C/min through heat of fusion. At -40°C plunge ampules into liquid nitrogen.
- 7. Ampules are stored in either the vapor or liquid phase of a nitrogen refrigerator.

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- 8. To establish a culture from the frozen state add 1.0 mL bacterized ATCC medium 802 to the frozen ampule and place it in a 35°C water bath. Immerse the vial to a level just above the surface of the frozen material. Do not agitate the vial.
- 9. Immediately after thawing, do not leave in water bath, aseptically remove the contents of the ampule and inoculate onto the surface of an ATCC medium 919 (non-nutrient agar) plate containing an overlay of 15.0 mL of bacterized ATCC medium 802.
- 10. Incubate at 25°C with the cap on loosely.
- 11. Once the culture is established, transfer 0.5 mL to 5.0 mL of bacterized ATCC medium 802.
- 12. Follow the protocol for maintenance of culture.

#### Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Oxytricha trifallax* (ATCC 50532)

#### References

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

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