



Product Sheet

# *Clostridium difficile* (ATCC® 9689™)

Please read this FIRST



Storage Temp.  
**Frozen: -80°C or colder**  
**Freeze-Dried: 2°C to 8°C**  
**Live Culture: See Propagation Section**

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Biosafety Level  
**2**

## Intended Use

This product is intended for research use only. It is not intended for any animal or human therapeutic or diagnostic use.

## Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Clostridium difficile* (ATCC® 9689™)

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

## Description

**Designation:** 90556-M6S [L.S. McClung 1780]  
**Deposited Name:** *Clostridium difficile* (Hall and O'Toole) Prevot  
**Genotype:** Toxinotype: 0  
**Product Description:** Type strain. Produces cytotoxin.

## Propagation

### Medium

ATCC® Medium 2107: Modified Reinforced Clostridial Agar/Broth Medium (pre-reduced)  
ATCC® Medium 260: Trypticase soy agar/broth with defibrinated sheep blood

### Growth Conditions

**Temperature:** 37°C  
**Atmosphere:** Anaerobic gas mixture, 80% N<sub>2</sub>-10% CO<sub>2</sub>-10% H<sub>2</sub>

### Propagation Procedure

1. Open vial according to enclosed instructions.
2. Under anaerobic conditions, withdraw 0.5 mL of the recommended broth from a single test tube (5 to 6 mL) and rehydrate the entire vial contents.
3. Aseptically transfer this aliquot back into the broth. Additional tubes may be inoculated with 0.5 mL each from the suspension. Also, 0.1 mL may be inoculated onto a slant. Streak several blood plates to check for colonial morphology and purity.
4. Incubate tubes under an anaerobic atmosphere at 37°C. Incubate one agar plate anaerobically for colony formation, and one aerobically for aerobic contamination check.
5. Within 48 hours, growth should be evident by turbidity in the broth and by colonies on the anaerobic agar surfaces. No growth occurs on agar plates incubated aerobically.

### ANAEROBIC CONDITIONS:

Anaerobic conditions for transfer may be obtained by either of the following:

- Use of an anaerobic gas chamber, or
- Placement of test tubes under a gassing cannula system connected to anaerobic gas.

Anaerobic conditions for incubation may be obtained by any of the following:

- Loose screw caps on test tubes in anaerobic chamber,
- Loose screw caps on test tubes in an activated anaerobic gas pack jar, or
- Use of sterile butyl rubber stoppers on test tubes so that an anaerobic gas headspace is retained.

## Notes

Anaerobe Systems Brucella Blood Plate (AS-111 or AS-141) can be used to analyze colony morphology and purity.

The presence of *tcdA* and *tcdB* genes is confirmed by PCR. The binary toxin gene *cdtB* is not amplified by PCR.

Purified genomic DNA of this strain is available as ATCC® 9689D-5™.

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

## References

References and other information relating to this product are available online at [www.atcc.org](http://www.atcc.org).

## Biosafety Level: 2

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

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the




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product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. 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 product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

### **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](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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