




Product Sheet


# *Clostridium difficile* (ATCC® 43255-FZ™)

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® 43255-FZ™)

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  
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## Description

**Designation:** VPI 10463  
**Deposited Name:** *Clostridium difficile* (Hall and O'Toole) Prevot  
**Genotype:** Toxinotype: 0  
**Product Description:** Used as toxinotype 0 reference strain. Ribotype 087.

## Propagation

### Medium

ATCC® Medium 5608: PY Medium (M2-VPI) With D-Glucose w/ Durham Tube  
ATCC® Medium 2107: Modified Reinforced Clostridial Agar/Broth Medium (pre-reduced)

### Growth Conditions

**Temperature:** 37°C  
**Atmosphere:** Anaerobic gas mixture, 80% N<sub>2</sub>-10% CO<sub>2</sub>-10% H<sub>2</sub> or 80% N<sub>2</sub>-20% CO<sub>2</sub>

### Propagation Procedure

1. Thaw the frozen vial under anaerobic conditions.
2. Under anaerobic conditions, transfer the entire contents of the thawed vial into a single tube (5 - 6 mL) of #5608 or #2107 broth. Mix well.
3. Additional tubes of broth may be inoculated with 0.5 mL each from the suspension and 0.1 mL may be inoculated onto a slant. Streak several blood plates for colony morphology and purity.
4. Incubate the tubes and plate under anaerobic conditions at 37°C. Incubate the purity plate aerobically at 37°C. Within 24-48 hours, growth should be evident by turbidity in the broth and by colonies on the anaerobic agar surfaces. No growth should occur 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

Colonies on anaerobic blood plates may appear erose, glistening, flat, and gray. Cells are Gram-positive straight rods. Agar growth takes between 24 to 48 hours.

Initial growth in #2107 may take between 24 to 48 hours, but after consecutive transfers, growth reaches peak viability and density between 6 and 12 hours. Better growth is observed on #5608.

The presence of both *tcdA* and *tcdB* genes have been confirmed by PCR. Toxin B production has been confirmed by cytotoxicity assay using Vero cells (Cell line # CCL-81). The binary toxin gene *cdtB* is not amplified by PCR

NOTE: Vial should be stored at -80C or lower until ready to be used. Storage in vapor phase liquid nitrogen is preferred for long term storage.

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

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