



Clostridioides difficile (Prevot) Lawson et al.

43255-FZ™

Description

Used as toxinotype 0 reference strain. Ribotype 087.

Strain designation: VPI 10463

Deposited As: *Clostridium difficile* (Hall and O'Toole) Prevot

Toxigenic: Yes

Toxin genes: *cdtB* (Binary toxin) negative; *tcdA* (Toxin A) positive; *tcdB* (Toxin B) positive

Storage Conditions

Product format: Frozen

Storage conditions: -80°C or colder

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 2

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 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 5608: PY Medium (M2-VPI) With D-Glucose w/ Durham Tube

ATCC Medium 2107: Modified Reinforced Clostridial

ATCC Medium 260: Trypticase soy agar/broth with defibrinated sheep blood

Temperature: 37°C

Atmosphere: Anaerobic

Handling Procedures

1. Thaw the frozen vial under anaerobic conditions.
2. Under anaerobic conditions, transfer the entire contents of the thawed vial

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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

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

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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Clostridioides difficile* (Prevot) Lawson et al. (ATCC 43255-FZ)

References

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

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Contact Information

ATCC

10801 University Boulevard

Manassas, VA 20110-2209

USA

US telephone: 800-638-6597

Worldwide telephone: +1-703-365-2700

Email: tech@atcc.org or contact your local distributor
