

***Clostridium difficile*, Isolate 4**

**Catalog No. NR-13430**

**For research only. Not for human use.**

**Contributor:**

NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH

**Product Description:**

Bacteria Classification: *Clostridiaceae*, *Clostridium*

Species: *Clostridium difficile*

Isolate: 4

Original Source: *Clostridium difficile* (*C. difficile*), isolate 4 was obtained from a human patient from the Mid-Atlantic region of the United States in 2008/2009.

*C. difficile* is a Gram-positive, spore-forming, obligate anaerobe that commonly inhabits the intestinal tract of various mammalian species, reptiles and birds, and may also be found in the environment. Pathogenic strains of *C. difficile* produce a potent cytotoxin (toxin B) and in most cases an enterotoxin (toxin A).<sup>1</sup> It is the production of these toxins in the gut which ultimately leads to the disease pseudomembranous colitis (PMC) and *Clostridium difficile* Associated Diarrhea (CDAD), usually occurring as a complication of antibiotic therapy in elderly hospitalized patients.<sup>2</sup>

**Material Provided:**

Each vial contains approximately 0.5 mL of bacterial culture in 0.5X Modified Reinforced Clostridial Broth supplemented with 10% glycerol.

Note: If homogeneity is required for your intended use, please purify prior to initiating work.

**Packaging/Storage:**

NR-13430 was packaged aseptically, in screw-capped plastic cryovials. The product is provided frozen and should be stored at -60°C or colder immediately upon arrival. For long-term storage, the vapor phase of a liquid nitrogen freezer is recommended. Freeze-thaw cycles should be avoided.

**Growth Conditions:**

Media:

Modified Reinforced Clostridial Broth ([ATCC medium 2107](#))  
Reinforced Clostridial Agar ([ATCC medium 1053](#)) or Tryptic Soy Agar (TSA) with 5% defibrinated sheep blood

Incubation:

Temperature: 37°C

Atmosphere: Anaerobic gas mixture  
(80% N<sub>2</sub>:10% CO<sub>2</sub>:10% H<sub>2</sub>)

Propagation:

1. Keep vial frozen until ready for use, then thaw.
2. Transfer the entire thawed aliquot into Reinforced

3. Clostridial Broth under anaerobic atmosphere.
3. Inoculate additional broth tubes with 0.5 mL each from the suspension. Slants may be inoculated with 0.2 mL each. Streak several Reinforced Clostridial Agar or TSA plates containing 5% defibrinated sheep blood to check for colony morphology and purity.
4. Incubate cultures at 37°C under anaerobic atmosphere for 48 to 72 hours.

**Citation:**

Acknowledgment for publications should read "The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: *Clostridium difficile*, Isolate 4, NR-13430."

**Biosafety Level: 2**

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2007; see [www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm](http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm).

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**References:**

1. Rupnik, M., M. H. Wilcox, and D. N. Gerding. "Clostridium difficile Infection: New Developments in Epidemiology and Pathogenesis." Nat. Rev. Microbiol. 7 (2009): 526-536. PubMed: 19528959.
2. Kelly, C. P. and J. T. LaMont. "Clostridium difficile - More Difficult Than Ever." N. Engl. J. Med. 359 (2008): 1932-1940. PubMed: 18971494.

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