

# Microbial DNA qPCR Multi-Assay Kit

## Clostridium difficile Pathogenicity

Cat. no. 330043 BBID-1506ZR-4

### For real-time PCR-based, application-specific microbial identification or profiling

The Clostridium difficile Pathogenicity Microbial qPCR DNA Multi-Assay Kit is a research tool used for the detection of virulence factors from C. difficile. This kit provides microbiology researchers with a convenient way to quickly detect virulence factor genes from C. difficile in isolated bacterial colonies, metagenomic samples, and other sample types. Virulence factors facilitate development of disease by allowing an infectious bacterium to establish itself on the host, in part by subverting or eluding host defenses, allowing attachment to the host, production of toxins, etc. This kit contains assays for detecting C. difficile itself as well as its virulence factor genes tcdB and tcdA. The assays use PCR amplification primers and hydrolysis-probe detection, increasing their specificity. The kit also contains Microbial qPCR Mastermix, Microbial DNA-Free Water, Microbial DNA Positive Control, and Positive PCR Control. The Positive PCR Control assay is included to test for the presence of PCR inhibitors and the efficiency of the polymerase chain reaction. Identification of whether pathogenic C. difficile is present in a sample and what virulence factors may be involved in its pathogenicity is accomplished using data analysis software with imported CT values generated by the PCR instrument software. The simplicity of the product format and operating procedure allow routine and reliable screening of pathogenic C. difficile from samples in any research laboratory with access to a real-time PCR instrument.

For further details, consult the *Microbial DNA qPCR Handbook*.

### Microbial DNA qPCR Multi-Assay Kits contents

Number of reactions	20 (25 µl PCR reactions)
Microbial DNA qPCR Assay BPID00110A	1 tube (20 µl)
Microbial DNA qPCR Assay BPVF00463A	1 tube (20 µl)
Microbial DNA qPCR Assay BPVF00464A	1 tube (20 µl)
Positive PCR Control (PPC)	1 tube (20 µl)
Microbial DNA Positive Control	1 tube (50 µl)

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<b>Number of reactions</b>	<b>20 (25 µl PCR reactions)</b>
Microbial DNA-Free Water	1 or 2 tubes (1.35 ml each), depending on number of assays
Microbial qPCR Mastermix	1 or 2 tubes (1.35 ml each), depending on number of assays



## Quality control data

Assay BPID00110A name	C. difficile
Assay BPID00110A No Template Control CT>35	Pass
Assay BPID00110A DNA Positive Control Template CT<34	Pass
Positive PCR Control CT = 22 ± 2	Pass
Microbial DNA Positive Control, CT<34	Pass
Microbial DNA-Free Water, free of contaminating DNA	Pass

Assay BPVF00463A name	tcdB
Assay BPVF00463A No Template Control CT>35	Pass
Assay BPVF00463A DNA Positive Control Template CT<34	Pass
Positive PCR Control CT = 22 ± 2	Pass
Microbial DNA Positive Control, CT<34	Pass
Microbial DNA-Free Water, free of contaminating DNA	Pass

Assay BPVF00464A name	tcdA
Assay BPVF00464A No Template Control CT>35	Pass
Assay BPVF00464A DNA Positive Control Template CT<34	Pass
Positive PCR Control CT = 22 ± 2	Pass
Microbial DNA Positive Control, CT<34	Pass
Microbial DNA-Free Water, free of contaminating DNA	Pass

## Shipping and storage

Microbial DNA qPCR Multi-Assay Kits are shipped on blue ice packs. For long-term storage, keep Microbial DNA qPCR Multi-Assay Kits at –20°C. Microbial qPCR Mastermixes are shipped on blue ice packs. For long-term storage, keep Microbial qPCR Mastermixes at –20°C. Microbial DNA-Free Water is shipped at ambient temperature or on blue ice packs. If unopened, Microbial DNA-Free Water can be stored at room temperature or at –20°C. If tube is opened, store Microbial DNA-Free Water at –20°C. Discard tube of Microbial DNA-Free Water if opened three times and use fresh tube of Microbial DNA-Free Water for future experiments.

**Note:** Ensure that you have the correct Microbial qPCR Mastermixes, with the correct reference dye if required, for your instrument.

**Note:** Open the package and store the products appropriately immediately upon receipt.

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# Assay Table

Assay #	Species (NCBI Tax ID)/Gene	NCBI Tax ID	Antibiotic classification / Gene Description	May detect (species) / Also detect (antibiotic resistance genes) / Associated species (virulence factor genes)	Sensitivity	Assay Catalog #
1	Clostridium difficile	1496			30	BPID00110A
2	tcdB		toxin B	Clostridium difficile	30	BPVF00463A
3	tcdA		toxin A	Clostridium difficile	20	BPVF00464A

# PCR plate loading sequence

Position	Species/gene	Template
A1	Clostridium difficile	NTC
A2	Clostridium difficile	Microbial DNA Positive Control
A3	Clostridium difficile	Sample 1
A4	tcdB	NTC
A5	tcdB	Microbial DNA Positive Control
A6	tcdB	Sample 1
A7	tcdA	NTC
A8	tcdA	Microbial DNA Positive Control
A9	tcdA	Sample 1
A10	Clostridium difficile	NTC
A11	Clostridium difficile	Microbial DNA Positive Control
A12	Clostridium difficile	Sample 2
B1	tcdB	NTC
B2	tcdB	Microbial DNA Positive Control
B3	tcdB	Sample 2
B4	tcdA	NTC
B5	tcdA	Microbial DNA Positive Control
B6	tcdA	Sample 2
B7	Clostridium difficile	NTC
B8	Clostridium difficile	Microbial DNA Positive Control
B9	Clostridium difficile	Sample 3
B10	tcdB	NTC
B11	tcdB	Microbial DNA Positive Control
B12	tcdB	Sample 3
C1	tcdA	NTC
C2	tcdA	Microbial DNA Positive Control
C3	tcdA	Sample 3
C4	Clostridium difficile	NTC
C5	Clostridium difficile	Microbial DNA Positive Control
C6	Clostridium difficile	Sample 4
C7	tcdB	NTC
C8	tcdB	Microbial DNA Positive Control
C9	tcdB	Sample 4
C10	tcdA	NTC
C11	tcdA	Microbial DNA Positive Control
C12	tcdA	Sample 4
D1	Clostridium difficile	NTC
D2	Clostridium difficile	Microbial DNA Positive Control
D3	Clostridium difficile	Sample 5
D4	tcdB	NTC
D5	tcdB	Microbial DNA Positive Control
D6	tcdB	Sample 5
D7	tcdA	NTC
D8	tcdA	Microbial DNA Positive Control
D9	tcdA	Sample 5
D10	Clostridium difficile	NTC
D11	Clostridium difficile	Microbial DNA Positive Control
D12	Clostridium difficile	Sample 6
E1	tcdB	NTC
E2	tcdB	Microbial DNA Positive Control
E3	tcdB	Sample 6
E4	tcdA	NTC
E5	tcdA	Microbial DNA Positive Control
E6	tcdA	Sample 6
E7	Clostridium difficile	NTC
E8	Clostridium difficile	Microbial DNA Positive Control
E9	Clostridium difficile	Sample 7
E10	tcdB	NTC

Position	Species/gene	Template
E11	tcdB	Microbial DNA Positive Control
E12	tcdB	Sample 7
F1	tcdA	NTC
F2	tcdA	Microbial DNA Positive Control
F3	tcdA	Sample 7
F4	Clostridium difficile	NTC
F5	Clostridium difficile	Microbial DNA Positive Control
F6	Clostridium difficile	Sample 8
F7	tcdB	NTC
F8	tcdB	Microbial DNA Positive Control
F9	tcdB	Sample 8
F10	tcdA	NTC
F11	tcdA	Microbial DNA Positive Control
F12	tcdA	Sample 8
G1	Clostridium difficile	NTC
G2	Clostridium difficile	Microbial DNA Positive Control
G3	Clostridium difficile	Sample 9
G4	tcdB	NTC
G5	tcdB	Microbial DNA Positive Control
G6	tcdB	Sample 9
G7	tcdA	NTC
G8	tcdA	Microbial DNA Positive Control
G9	tcdA	Sample 9
G10	Empty	Empty
G11	Empty	Empty
G12	Empty	Empty
H1	PPC	Sample1
H2	PPC	Sample2
H3	PPC	Sample3
H4	PPC	Sample4
H5	PPC	Sample5
H6	PPC	Sample6
H7	PPC	Sample7
H8	PPC	Sample8
H9	PPC	Sample9
H10	Empty	Empty
H11	Empty	Empty
H12	Empty	Empty

## Ordering Information

Product	Contents	Cat. no.
Microbial DNA qPCR Multi-Assay Kit	One 20 $\mu$ l tube for each Microbial DNA qPCR Assay, 1 tube Positive PCR Control (20 $\mu$ l), 1 tube Microbial DNA Positive Control (20 $\mu$ l), 1 or 2 tubes Microbial DNA-Free Water (1.35 ml each), 1 or 2 tubes Microbial qPCR Mastermix (1.35 ml each)	330043

Microbial DNA qPCR Multi-Assay Kits are intended for molecular biology use only. These products are not intended for the diagnosis, prevention, or treatment of a disease.

Learn more at [www.qiagen.com](http://www.qiagen.com)!

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