Igenesis CE IVD

FOR PROFESSIONAL USE ONLY

Toxigenic Clostridium difficile Gene (toxinA, toxinB) Detection Kit (PCR Fluorescence)

REF 106-0159-01

Product Name
Toxigenic Clostridium difficile Gene (toxinA, toxinB) Detection Kit (PCR Fluorescence)

Specification
12 reactions/kit

Intended Use
This kit adopts multiplex TaqMan fluorescent quantitative PCR technology for the qualitative in vitro detection of *Clostridium difficile* toxin A gene (*tcdA*) and toxin B gene (*tcdB*) in human fecal samples. The test results can aid in the diagnosis of toxigenic *Clostridium difficile* infections.
Clostridium difficile (CD), also known as *Clostridioides difficile* or *C. diff*, is a Gram-positive, obligately anaerobic, spore-forming bacillus. It produces spores and is widely distributed in the environment, as well as in the intestines of mammals and humans. *Clostridium difficile* infection (CDI) primarily arises from antibiotic abuse or compromised immune systems, which can lead to the overgrowth of this opportunistic pathogen. This overgrowth may result in symptoms such as diarrhea, colitis, pseudomembranous colitis, and, in severe cases, even death.
Toxigenic *Clostridium difficile* primarily produces three toxins: toxin A, toxin B, and binary toxin. Toxin A and toxin B are encoded by the *tcdA* and *tcdB* genes, respectively. Toxin A primarily acts within the intestinal epithelium and is thus termed an enterotoxin, while toxin B exhibits broader cellular tropism and is referred to as a cytotoxin.

Test Principle
The Automated Fully Enclosed qPCR Instrument, uses Intelligent cassette (iCassette) technology to automatically perform nucleic acid extraction, amplification, data reading and result analysis. The instrument includes two parts: nucleic acid extraction and multiple fluorescent PCR. At the same time, the built-in QR code scanner can automatically identify the execution process corresponding to this kit. The uniquely designed software is used to execute the extraction and PCR process of the kit, display the results and analyze the results. The kit contains 12 disposable iCassettes. The reagents prefilled in the iCassette include nucleic acid extraction reagents and PCR reaction reagents, which can be performed on the Automated Fully Enclosed qPCR Instrument, Since the Automated Fully Enclosed qPCR Instrument is fully enclosed and automatically performs nucleic acid extraction and PCR reactions, it reduces direct cross-contamination of samples. For a complete description of the instrument, please refer to the user manual of the corresponding instrument.
This kit utilizes real-time multiplex fluorescent PCR technology to detect *Clostridium difficile* toxin A gene (FAM labeled) and toxin B gene (Texas Red labeled) as target regions. Specific primers and fluorescent probes are designed for each target to perform PCR amplification. Additionally, primers and probes targeting an internal control (HEX labeled) are included to confirm adequate processing of the target bacterial strain and detect the presence of PCR reaction inhibitors through detection of the control sample.

Components

Kit Components			Content	Quantity
CD iCassette (12 pcs)	Nucleic acid extraction reagent	Magnetic beads	Magnetic microsphere	11μL/iCassette
		CD lyophilization A	Proteinase K	1pc/iCassette
		CD lyophillization B	Bacillus atrophaeus	1 pc/iCassette
		Virus lysate solution	Guanidine isothiocyanate	800μL/iCassette
		Virus binding solution	Guanidine isothiocyanate	200μL/iCassette
		Virus washing solution	Sodium chloride	900μL/iCassette
		Virus eluent	Tris-HCl	90μL/iCassette
	CD PCR reagent	CD PCR cosolvent solution	5×RT Buffer	35μL/iCassette
		CD lyophillization	Specific primer probe, dNTP, enzyme	1 pc/tube
Control materials	CD positive control		Pseudovirus containing target fragments	1tube(1000μL)
	CD negative control		Sterilized purified water	1tube(1000μL)

Note:Components in kits with different batch numbers are not interchangeable

Storage condition and Shelf life
1. The kit can be stored at 2-8℃ and the shelf life is 9 months.
2. The transportation temperature range of the kit should be kept at 2-8℃.
3. Please do not open the iCassette cover before adding the sample. If you open the iCassette cover, it should be used within 30 minutes.

Applicable instruments
Automated Fully Enclosed qPCR Instrument:Galaxy Nano,Galaxy Neo, Galaxy Lite and Galaxy Pro.

Materials Required but Not Provided
- Automated Fully Enclosed qPCR Instrument : Galaxy Nano,Galaxy Neo,Galaxy Lite or Galaxy Pro.
- Ultrosonic machine: Galaxy USL III *(not required for Galaxy Neo)*.
- Leak-proof, sterile, screw-capped specimen collection containers.
- Disposable gloves, eye protection, laboratory coats, and labels or permanent marking pen.
- Pipettes.
- Sterile pipette tips.

Sample Requirements
1. Sample types:Stool.
2. Sample collection

Have the patient naturally defecate into a dry, clean bedpan or disposable container. Using a sampling spoon/stick, collect 4-6g of the specimen from different areas of the stool (particularly from the mucous or purulent/bloody portions), avoiding contact with the edges of the bedpan. For liquid stool, collect ≥5ml. Immediately transfer the sample into a sterile container or use a transport tube containing preservative solution. If using a preservative tube, ensure the stool is thoroughly mixed into the liquid.

3. Sample storage and transportation

Specimens to be tested can be immediately processed, specimens to be tested within 24 hours can be stored at 2~8°C. Specimens that cannot be detected within 24 hours should be stored at-70°C or below (in the absence of -70°C storage conditions, specimens to be tested can be stored at -20°C for 10 days). Multiple freeze/thaw cycles should be avoided. Specimens should be transported in a sealed frozen container with ice or in a sealed foam box with ice packs.

4. Principles of Biosafety Protection:

All operations should comply with relevant national laws and regulations.

Test Method and Operation
1. Prepare the CD iCassette

1.1 Open the package of CD lyophilization, observe whether the CD lyophilization is intact, replace the empty PCR tube on the iCassette with the PCR tube containing the CD-F lyophilization, and make sure the PCR tube is screwed up.
1.2 Processing samples or control materials in the samples preparation room,first vortex the collection tube for 10-15 seconds, for fecal samples, it is recommended to use Igenesis's pre-treatment consumables (Sniffer CP Trace Sample DNA Extraction kit) for sample processing. Specific operational steps should be followed as detailed in the corresponding product manual.
1.3 Open the lid of the iCassette, and pipette 1000 μl of sample or controls to the CD iCassette sample compartment as shown in Figure 1. slowly, close the lid tightly, put it into the ultrasonic machine, and sonicate for 20 seconds. (Note: After the sample is added to the iCassette, the test needs to start running within 30 minutes)
1.4 Place the iCassette into the instrument.

Note: For the Galaxy Neo, the ultrasound functionality is already built-in, so simply add the sample to be tested or controls into the iCassette and insert it into the machine.

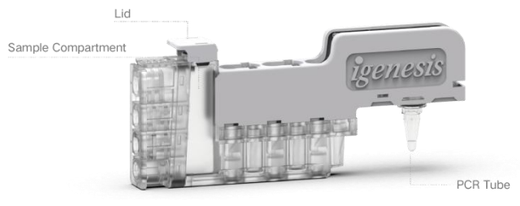

Figure 1. CD iCassette (Lateral View)

2. Test Operation

2.1 Turn on the power, press the switch button on front side of the instrument, and the blue light is on, i.e. "the instrument is on". After the instrument is turned on, click the control software icon on the desktop to enter the login interface.
2.2 Log in to the software for the first time with the administrator account (Admin/123456), click "OK" to complete the login.
2.3 Click the "Open" button in the initial interface to open the compartment door of the instrument.
2.4 Put the iCassette that has been added with sample into the instrument and click the "Close" button in the initial interface to close the compartment door. (For multi-channel instrument, iCassette tray holder is required to load the iCassette. Input the sample information at the corresponding channel position of the control software while loading the iCassette)
2.5 After the compartment door is closed, the instrument scans the corresponding QR code on the iCassette automatically.
2.6 After scanning, click the "Run" button, the software will automatically load the program corresponding to the QR code of the iCassette, and click "OK" to start the program.
2.7 After the program starts , the progress of the instrument running will be displayed in the main interface.
2.8 After the amplification is completed, the compartment door will open automatically.
For the detailed steps of test operation, please refer to user manual of the instrument.

3. Result Analysis

After the experiment is finished, the instrument will automatically save the results, and output the Ct value and amplification curves with results interpretation in the interface.

Quality Control
Internal control: When the sample test result is negative, the internal control HEX channel must be positive;
Probe quality control: if the fluorescence signal measurement of the probe displays "probe check fail", it indicates that there is a problem with the probe in the iCassette and the iCassette is unavailable; If the PCR step is performed after the iCassette self-test, it indicates that the iCassette probe is qualified.
The above requirements must be met at the same time in the same experiment, otherwise, this experiment is invalid and needs to be repeated.
Test results of positive control: FAM channel, HEX channel and Texas Red channel are all positive.
Test results of negative control: FAM channel and Texas Red channel are all negative,HEX channel is positive.

Reference Interval
Based on the analysis of clinical sample test results, using the ROC curve method, the final determined Ct positive threshold value for this kit is 37.

Interpretation of Test Results
The sample to be tested is judged according to the standards in the table below：

FAM Channel	HEX Channel	TEXAS RED Channel	Results
√	√/×	×	toxin A gene positive(Figure 2)
×	√/×	√	toxin B gene positive (Figure 3)
√	√/×	√	toxin A gene and toxin B gene positive
×	√	×	Negative sample (Figure 4)
√	√	√	Positive control
×	√	×	Negative control
×	×	×	Invalid, repeat the test

Note：“√" means that the result "has an obvious logarithmic amplification curve"；"×" indicates the result "no logarithmic amplification curve"."√/×" means that the result "may be positive or negative". The HEX channel is an internal standard channel. Because of its specific competition with the sample, when the test sample is positive, it may be tested negative.

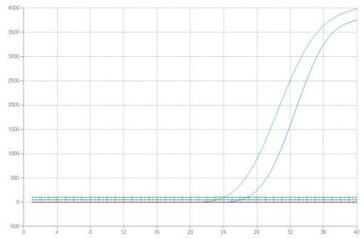

Figure 2. toxin A gene positive

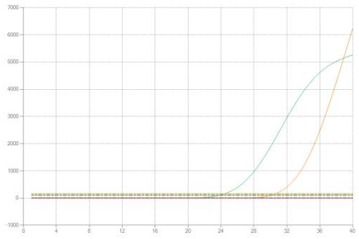

Figure 3. toxin B gene positive

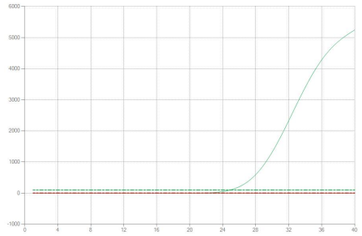

Figure 4.Negative

Limitations of Test Method

1. The test results of this kit are for clinical reference only. The clinical diagnosis and treatment of patients should be considered in combination with their symptoms/signs, medical history, other laboratory tests, and treatment response.
2. Improper sample collection, handling, transportation, and storage conditions, as well as a low viral titer in the sample, may lead to false-negative results.
3. Unverified cross-reacting substances may cause false-positive results.
4. Other unverified interferences, such as endogenous or exogenous substances introduced into the sample, may result in false-negative results.
5. Cross-contamination between samples may lead to false-positive results.
6. This kit specifically targets *tcdA* and *tcdB* genes and does not detect additional C. difficile virulence factors (e.g., *cdtA/cdtB*, binary toxin genes).
7. This assay is validated solely for fecal specimens. Results do not distinguish colonization, contamination, or true pathogenicity. Clinical correlation is required.

Product Performance Index

1. The LoD of this kit: The LoD by number of plasmid copies is 10^3 copies/mL, while that determined by bacterial culture method is 10^3 CFU/mL.
2. This test kit has no cross-reactivity with *Salmonella, Shigella, Campylobacter jejuni, Escherichia coli (E. coli), Staphylococcus aureus, Yersinia enterocolitica, Vibrio parahaemolyticus, Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae, or related species.*
3. The intra-batch precision coefficient of variation (CV%) is ≤5%.

Precautions

1.If the iCassette is oscillated after adding the sample, do not use the iCassette.
2.Clinical laboratories should strictly follow the management standards in the local related regulations for molecular biology laboratories and clinical gene amplification laboratories.
3.Each iCassette is single-use, please do not reuse it.
4. In the sample processing stage, use a negative pressure ultra-clean workbench.
5. During the experiment, the medical staff must wear work clothes, disposable gloves, and use self-discharging pipettes.
6. After the experiment, the workbench and pipette were treated with 2% sodium hypochlorite or 75% alcohol, and then irradiated with a UV lamp for 30 minutes.

References

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2. VOTH D E, BALLARD J D. Clostridium difficile toxins: mechanism of action and role in disease[J]. Clinical Microbiology Reviews, 2005, 18(2): 247-263.
3. PAN Junfei,ZHANG Aijun,LI Gang,et al.Preparation of antibodies against epitopes of Clostridium difficile toxin B and establishment of a sandwich ELISA technique[J].Journal of Pathogen Biology,2020,15(3):285-290+297.
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5. YIN Lunlun,WAN Wang,DENG Zhongping,TIAN Xiangrong,ZHOU Qiang,REN Xiaomei.Establishment and application of a multiplex fluorescent PCR detection method for toxigenic Clostridium difficile[J/OL].Research in Life Sciences. https://doi.org/10.16605/j.cnki.1007-7847.2024.01.0118.

Instruction Version

Version: A/1
Date of Issue: March,2022
Last revised: May, 2025

Symbols

The following symbols may appear on the product labeling:

IVD	In vitro diagnostic medical device		Do not re-use
	Use-by date		Consult instructions for use or consult electronic instructions for use
	Caution		Manufacturer
	Temperature limit	LOT	Batch code
	Contains sufficient for <n>		Keep dry
	Keep away from sunlight		Do not use if package is damaged and consult instructions for use
	Date of manufacture		Biological risks
REF	Catalogue number	CE	CE marking of conformity
EC REP	Authorized representative in the European Community		

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