Toxigenic Clostridium difficile DNA Diagnostic Kit (PCR-Fluorescence Probing)

Reference Number

S3350E-12-P, S3350E-24, S3350E-48, S3350E-12-S

Product Name

Toxigenic Clostridium difficile DNA Diagnostic Kit (PCR-Fluorescence Probing)

Package Specification

Pre-packaged 12 tests/kit, 24 tests/kit, 48 tests/kit

Intended Use

The Toxigenic Clostridium difficile DNA Diagnostic Kit (PCR-Fluorescence Probing) uses real-time fluorescent PCR technology to qualitatively detect the conserved gene sequences of toxigenic *clostridium difficile* nucleic acid in the feces of suspected patients. The results are used to aid in the diagnosis of toxigenic *clostridium difficile* infection and should not be used as the sole basis for patient management decisions.

This kit is designed to be used by professional, qualified, and trained clinical laboratory personnel who have received special guidance and training in real-time PCR and *in vitro* diagnostic procedures.

For in vitro diagnostic use only. For professional use only.

Summary

Generally, *Clostridium difficile* DNA can be detected in the specimens of patients with food-borne infections, and a positive result indicates the presence of toxigenic *Clostridium difficile* DNA; confirmation of the patient's infection status needs to be combined with clinical medical history and other diagnostic information. A positive result does not exclude other viral infections or co-infection with other bacteria. The detected pathogen may not be the exact cause of the disease. A negative result does not exclude *Clostridium difficile* infection. Negative results must be combined with clinical observation, patient history, and epidemiological information.

Test Principle

The Toxigenic Clostridium difficile DNA Diagnostic Kit (PCR-Fluorescence Probing) is a real-time polymerase chain reaction (qPCR) test. The primers and probes set are designed to detect *Clostridium difficile* DNA in feces samples of patients with toxigenic *Clostridium difficile* infection. This kit is used to qualitatively detect the conserved gene sequences of toxigenic *Clostridium difficile* DNA.

An exogenous internal control for monitoring sample processing and qPCR process to avoid false negative results.

Components of the Diagnostic Kit

This kit is an amplification reaction reagent and contains the following components:

No.	Reagent Name	Spec. & Qty.			Main Ingredients
		24 T	48 T	Pre-packaged 12T	
1	TCD PCR Mix	864 μL/ tube x 1 tube	864 μL/ tube x 2 tubes	36 μL/ tube x 12 tubes	Primers, Probes, dNTPs, $MgCl_2$, PCR buffer
2	TCD PCR Enzyme Mix	96 μL/ tube x 1 tube	192 μL/ tube x 1 tube	4 μL/ tube x 12 tubes	Taq DNA polymerase, UNG Enzyme
3	TCD Positive Control	1000 μL/ tube x 1 tube	1000 μL/ tube x 1 tube	1000 μL/ tube x 1 tube	The plasmid containing target genes
4	TCD Negative Control	1000 μL/ tube x 1 tube	1000 μL/ tube x 1 tube	1000 μL/ tube x 1 tube	Normal saline
5	TCD Internal Control	500 μL/ tube x 1 tube	500 μL/ tube x 1 tube	5 μL/ tube x 12 tubes	The plasmid containing IC gene

Note:

1. All contents in this package are prepared and validated for the intended testing purpose. Replacement or modification of any of the package contents will affect the testing performance of the kit. Components contained within a kit are intended to be used together. Do not mix or exchange components from different kits.
2. All biological materials in this diagnostic kit have been inactivated.
3. Materials required but not provided: 1.5 mL DNase-free and RNase-free microfuge tubes; 0.2 mL PCR reaction tubes or strip; Various models of pipettes and pipette tips (10 μL, 200 μL and 1000 μL tips with filters); Microcentrifuge; Vortex mixer.
4. Reagent required but not provided:Sample Release Reagent (Reference Number: S1014E Series) or Nucleic Acid Extraction-Purification Kit (Reference Number: S50016E-12A) manufactured by Sansure Biotech Inc. for nucleic acid extraction.

Precautions

1. For *in vitro* diagnostic use only. Please read the product manual carefully before operation.
2. Please learn and be familiar with the operation procedures and precautions for each instrument before test. Please make sure quality control is performed for each test.
3. Laboratory management shall strictly follow management practices of PCR gene amplification laboratory, laboratories personnel must receive professional training, test processes must be performed in separated regions, all consumables should be for single use only after sterilization, special instruments and devices should be used for every process, all lab devices required in different processes and regions should not be cross-used.
4. All samples for detection should be handled as potentially infectious. Wear laboratory coats, protective disposable gloves and change the gloves often to avoid cross-contamination between samples. Handling of samples and waste must meet relevant requirements outlined in local, state and national regulations.
5. Note: Improper operation during the storage, transportation and use of the reagent may affect the test results. For example, improper storage and transportation, sample collection, sample processing and test process are not standardized. Please strictly follow the instructions.
6. Due to the characteristics of swab and other sample collection process and viral infection process itself, false negative results may be caused by insufficient sample volume, which should be combined with other clinical diagnosis and treatment information for comprehensive judgment, retest when necessary.
7. After the addition of the sample in the tube the resulting solution is to be considered potentially biohazardous, handle the reagent with appropriate precautions and good laboratory practice.
8. The safe disposal of the reagents supplied must be carried out according to the instruction contained in the specific Safety Data Sheets and in compliance with the national regulations on disposal of potentially hazardous waste.
9. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established; If you have any questions about the test or the results, please contact Sansure`s customer service hotline +86-731-88883176-6116 or send an email to info@sansure.com.cn/ support@sansure.com.cn.

Storage and Stability

1. The shelf life of the kit is 12 months at -25°C to -15°C and protected from light.
2. Please refer to the date of manufacture and expiry date on the outer package.
3. The reagents keep valid and stable within the expiry date if not used. As long as the container of the reagent is opened, the freeze/thaw cycles should not exceed three.

Compatible Instrument

The kit is compatible to Fluorescence Quantitative Analysis System containing FAM, VIC, ROX and CY5 channels such as:

1) Applied Biosystems/7500 Real-Time PCR System;
2) Applied Biosystems/QuantStudio 5 Dx Real-Time PCR System.
3) Bio-Rad/CFX96 Dx and CFX96 Deepwell Dx Systems;
4) Hongshi/SLAN®-96P Real-Time PCR System;
5) Molarray/Real-Time Quantitative Thermal Cycler (Model: MA-6000);
6) Sansure/Portable Molecular Workstation (Model: S-Q36A);
7) Sansure /Portable Molecule Workstation (Model: S-Q31A/S-Q31B).
8) Sansure/Portable Molecular Diagnostic System (S-Q37A/S-Q37B）.

Specimen Requirements

1. Applicable specimen type: feces.
2. Collection of specimen:

Feces: For patients with gastrointestinal symptoms such as diarrhea at the early stage of the disease, preserve 3~5 g (soybean size) feces. The specimen collection tube should be pasted with the barcode first. Collect sample into specimen collection tube with screw cap containing 2mL normal saline then seal with sealing film. After sample collection, it is recommended to place into Sample Storage Reagent for preservation. If necessary, it is recommended to use Multi-type Sample DNA/RNA Extraction-Purification Kit (Magnetic beads method) (Reference Number: S50016E Series) manufactured by Sansure Biotech Inc. for nucleic acid extraction.

3. Storage and delivery of specimens:

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, nucleic acid can be stored at -25°C to -15°C for 15 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.

Test Method

1. Please process according to the following steps for SLAN®-96P Real-Time PCR System, 7500 Real-Time PCR System, QuantStudio 5 Dx Real-Time PCR System, Real-Time Quantitative Thermal Cycler (Model: MA-6000), CFX96 Dx and CFX96 Deepwell Dx Systems instrument:

1.1 Preparation of reagent (performed at "reagent preparation room")

1.1.1 Take out all the components out off the kit and equilibrate them at room temperature, then vortex each of them respectively for later use.

1.1.2 Prepare the TCD PCR Master Mix according to following table. The volume required is based on the total number of specimens, plus a TCD Positive Control and a TCD Negative Control. Mix thoroughly then centrifuge it for later use. The remaining reagent must be stored at -20°C immediately.

	1 sample	10 samples	24 samples	48 samples
TCD PCR Mix	36 μL	360 μL	864 μL	1728 μL
TCD PCR Enzyme Mix	4 μL	40 μL	96 μL	192 μL

Note: The above configuration is for reference only.

1.2 Processing and loading of specimens (performed at "specimen processing room")

1.2.1 Use the Nucleic Acid Extraction-Purification Kit (Reference Number: S50016E Series) manufactured by Sansure Biotech Inc. to extract nucleic acid. Add 5 μL of TCD Internal Control to 300 μL of the samples to be tested (in the ratio of 5:300) and mix thoroughly to form the mixed solution. Centrifuge at 2000rpm for 10s and then set aside. The Negative and Positive Controls in the kit participate in the extraction.

1.2.2 Add 10 μL of the extracted RNA to the PCR tubes in the following order: TCD Negative Control, patient specimen(s), and TCD Positive Control.

1.2.3 Add 40 μL of TCD PCR Master Mix into each well. Cover each well, centrifuge at 2000 rpm for 10 seconds, and place into the Real-Time PCR system.

1.3 PCR Amplification (Refer to user manual of each instrument to adjust the settings.)

1.3.1 Place PCR reaction tubes into the specimen wells of the amplification equipment. Set up the TCD Positive Control,TCD Negative Control and specimens to be tested in order and input specimen name.

1.3.2 Select PCR test channel:

1) Select FAM channel to test *tcd*A gene, select ROX channel to test *tcd*B gene, and select VIC channel to test *cdt*A gene.

2) Select CY5 channel to test internal control.

1.3.3 Set cycle parameters

	Steps	Temperature	Time	Cycle No.
1	De-contamination	50°C	2 min.	1
2	DNA pre-denaturation	94°C	5 min.	1
3	Denaturation	94°C	15 sec.	45
	Annealing, extension and fluorescence collection	60°C	30 sec*.	

*Note: Due to ABI 7500's technical specification, it can not be set at 30 sec., but at 31 sec. or 32 sec.

When the settings are completed, save the settings and carry out the reaction procedure.

2.Please process according to the following steps for Portable Molecule Workstation (Model: S-Q31A/S-Q31B):

2.1 Preparation of consumables and reagents

(1) Take out the reaction tube carrier, PCR reaction tube and Tip.

(2) Put the **Tip** into Well H,and **PCR reaction tube** into **Well** PCR.(The well location information has been marked on the reaction tube carrier)

(3) Put Sample Release Reagent (Reference Number : S1014E Series) into the **Well** B; Put TCD PCR Mix into the **Well** C; Put TCD PCR Enzyme Mix into the **Well** D; Put internal control into the **Well** A.

(4) Add 20 μL sample to be tested or TCD Positive Control or TCD Negative Control into the **Well** B (To avoid bubbles during operation, it is recommended to pipet deeply and release slowly).

2.2 Test Procedure (Refer to user manual of each instrument to adjust the settings.)

2.2.1 Gently press the front door to open it.

2.2.2 Place the **Well** A of reagent strip into the instrument towards the outside of the instrument,and close the front door of the instrument.

2.2.3 Click the**"Experimental task"** on the instrument display screen to enter the interface of setting new experimental task.

2.2.4 Select the required experimental project in the drop-down menu of **Experimental project**, enter the corresponding task name in the **Task Name** bar, and input and select other items that should be input or selected.

2.2.4 Click **"Submit"** to submit the experimental task and **"OK"** to run the instrument and start the experimental task successively.

2.2.5 When the Portable Molecule Workstation (Model: S-Q31B) shows **"Please transfer the PCR tube to the 1/2/3/4"**(The S-Q31A shows "Please transfer the PCR tube") on the interface, take out the PCR tube and cover it well, then centrifuge it instantaneously.

2.2.6 Insert the PCR tube into the PCR amplification module (the "PCR 1/2/3/4" cover has been automatically opened at this time), close the PCR lid of the amplification module, then click

"OK" for amplification detection.

3.Please process according to the following steps for Portable Molecular Workstation (Model: S-Q36A):

3.1 Preparation of consumables and reagents

(1) Take out the Consumables kits and reagents.

(2) Put Sample Release Reagent (Reference Number : S1014E Series) into the **Well** B; Put TCD PCR Mix into the **Well** C; Put TCD PCR Enzyme Mix into the **Well** D; Put internal control into the **Well** A ,(The well location information has been marked on the carrier set)

(3) Add 20 μL sample to be tested or TCD Positive Control or TCD Negative Control into the **Well** B (To avoid bubbles during operation, it is recommended to pipet deeply and release slowly).

3.2 Test Procedure (Refer to user manual of each instrument to adjust the settings.)

3.2.1 Click the "and" button on the instrument display screen to open the door of the instrument and put the prepared consumables into the designated position of the instrument.

3.2.2 Click the "New" on the instrument display screen to enter the new experiment task setting interface.

3.2.3 Select the required experimental project in the drop-down menu of Experimental project, enter the corresponding task name in the Task Name bar, and input and select other items that should be input or selected.

3.2.4 Click "Submit" to submit the experimental task and "OK" to run the instrument.

4. Please process according to the following steps for Portable Molecular Diagnostic System (S-Q37A/S-Q37B):

4.1 Pre-run preparation

4.1.1 Load the amplification reagent component assembly into the extraction reagent component (Nucleic Acid Extraction-Purification Kit, Reference Number: S50016E-12A) to compose the test reagent cartridge;

4.1.2 Open the seal plug of the sample loading hole, add 350μL sample or TCD Positive Control or TCD Negative Control into the sample loading hole (To ensure Diagnostic System have 300uL samples for nucleic acid extraction); or pipette sample by transfer pipet included in the extraction reagent component into the sample loading hole (When sample enter the lower bubble of transfer pipet indicating enough sample has been taken). Then close the seal plug.

4.2 Test Procedure

4.2.1 Click the "Specimen" button on the instrument display screen to open the door of the instrument and enter the new experiment task setting interface.

4.2.2 Put the prepared consumables into the designated position of the instrument.

4.2.3 Enter specimen information, select the required experimental project in the drop-down menu of Experimental project, enter the corresponding task name in the Task Name bar, and input and select other items that should be input or selected.

4.2.4 Click "Submit" to submit the experimental task and "OK" to run the instrument

5. Result Analysis (Refer to user manual of instrument to adjust the settings.)

Results will be saved automatically when reactions are completed. Analyze amplification curve of target of detection and internal control. Adjust Start, End and Threshold values of Baseline of the graph according to analysis result (Users can adjust the values according to the actual situation. Start value can be set between 3-15, and End value between 5-20. Adjust the amplification curve of negative control to be flat or below threshold). Click "Analyze" to implement the analysis, make sure each parameter satisfies the requirements given in "5. Quality Control". Go to "Plate" window to record qualitative results.

6.Quality Control

All test controls should be examined prior to interpretation of patient results. If the controls are not valid, the patient results cannot be interpreted. The Ct cutoff value of this kit is set as 40 and the end user is required to review fluorescent curves before final interpretation. All positive curves should be typical S-shape amplification curves or without plateau for weakly positive samples.

6.1 Negative Control: FAM, ROX and VIC channels has no Ct value display or FAM, ROX and VIC channels Ct value> 40, CY5 Ct value ≤ 35;

6.2 Positive Control: FAM, ROX, VIC and CY5 channels show typical S-shape amplification curves, FAM, ROX, VIC and CY5 Ct values are ≤ 35;

6.3 The above requirements must be met at the same time in the same experiment, otherwise, this experiment is invalid and needs to be repeated. If there is contamination for the re-run, please perform decontamination procedures.

Reference Range

Through the research on reference values, the Ct cutoff value of target gene is determined to be 40, the Ct cutoff value of internal control is determined to be 40.

Explanation of Detection Result

Assessment of clinical specimen test results should be performed after the positive and negative controls have been examined and determined to be valid and acceptable. If the controls are not valid, the patient results cannot be interpreted. The table below describes the result interpretation concerning the use of the controls mentioned above. The end user is required to review fluorescent curves before final interpretation. All the positive curve should be typical S-shape amplification curve or without plateau for weakly positive samples (38≤Ct≤40).

*tcd*A (FAM)	*tcd*B (ROX)	*cdt*A (VIC)	IC (CY5)	Results
+	Not considered	Not considered	Not considered	Toxigenic *Clostridium difficile* Positive
Not considered	+	Not considered	Not considered	Toxigenic *Clostridium difficile* Positive
Not considered	Not considered	+	Not considered	Toxigenic *Clostridium difficile* Positive
-	-	-	+	Toxigenic *Clostridium difficile* Negative
-	-	-	-	Invalid

Result of (-): Ct value >40 or Undetermined; Result of (+): Ct value ≤ 40.

Invalid Result: There is no typical S-shape amplification curve or Ct > 40 or No Ct detected for *tcd*A (FAM), *tcd*B(ROX), *cdt*A(VIC) and internal control (CY5), indicating that the specimen concentration is too low, or there are interfering substances that inhibit the reaction. If upon retest, the result is invalid again, another fresh sample should be collected and tested.

For technical assistance of test results, please contact local distributor or email: info@sansure.com.cn.

Limitations of Detection Method

1. False negative results can be caused by poor specimen quality, improper sample collection, improper transportation, improper laboratory processing, or a limitation of the testing technology.
2. Mutation in the target sequence of toxigenic *Clostridium difficile* or change in the sequence due to other reasons may lead to false negative results.
3. Improper reagent storage may lead to false negative results.
4. Use of this assay is limited to personnel who are trained in the procedure.
5. Test results of the diagnostic kit can only be used as an aid in clinical diagnosis. Symptoms and physical signs, disease history, other laboratory examinations and therapeutic reactions of the patients should be comprehensively considered for the clinical diagnosis and treatment.
6. Unverified interfering substances or PCR inhibitors may lead to false negative or invalid results.

Product Performance Index

1. Specificity

This kit is compatible with *Norovirus*, *Herpes simplex virus* type 1 / 2, *Enteroadenovirus*, *Varicella zoster virus*, *Rotavirus*,*Hepatitis A virus, Stellate virus, EB virus, Rubella virus, Measles virus, Influenza A virus, Parainfluenza virus, influenza B virus, Cytomegalovirus, Respiratory syncytial virus, Mumps virus*, *Hepatitis E virus*, Group B *streptococcus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Neisseria meningitidis*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Salmonella*, *Shigella*, *Vibrio parahaemolyticus*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus viridis*, *Neisseria*, *Rhodobacteria mucus* and *Proteus* had no cross reaction. The negative reference materials of the enterprise were tested and the results were negative.

2. Limit of detection

The limit of detection of this kit is 500 copies/mL.

3. Precision

The coefficient of variation (CV,%) of Ct value of the precision is ≤ 5%.

4. Possible interfering substances in specimens

100 μg/mL oxymetazoline hydrochloride, 50 μg/mL dexamethasone, 50 μg/mL cefmenoxime hydrochloride, 100 μg/mL oseltamivir, 100 μg/mL zanamivir, 100 μg/mL acetaminophen Bavirin, 100 μg/mL azithromycin, 200 μg/mL histamine hydrochloride, 50 μg/mLL beclomethasone, 50 μg/mL benzocaine, 50 μg/mL mupirocin, 50 μg/mL Bromycin, 100 μg/mL phenylephrine, 50 μg/mL mometasone, 50 μg/mL fluticasone, 100 μg/mL peramivir, 50 μg/mL budesonide, 100 μg/mL triamcinolone acetonide, 10 μg/mL flunisolide, 60 μg/mL sodium chloride, 100 μg/mL urea, 10 μg/mL heme, 20 μg/mL purified mucin, 100 μg/mL FeCl3, 20% (v/v) absolute ethanol, and 20% (v/v) human whole blood have no significant interference with the detection results of the kit.

Bibliography

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3. Bartlett G. Narrative Review: the New Epidemic of Clostridium difficile-associated Enteric Disease[J]. Ann Intern Med. 2006 , 21;145(10):758-64.

Symbols

Symbols	Meanings	Symbols	Meanings
IVD	*In Vitro* Diagnostic Medical Device	LOT	Batch Code
	Use-by date	REF	Reference Number
	Manufacturer		Date of Manufacture
Σ	Contains sufficient for <n> tests		Temperature Limit
	Caution		Consult Instructions for Use
21 PAP	PAP21: Not corrugated cardboard		Do not re-use
EC REP	Authorized representative in the European Community	CE	This product fulfills the requirements of the European Directive 98/79/EC for *in vitro* diagnostic medical devices.
PCR MIX	PCR Mix	ENZYME MIX	Enzyme Mix
INTER CONTROL	Internal Control	VERSION	Version
CONTROL -	Negative Control	CONTROL +	Positive Control
PRE	Prepackaging		Keep away from light
UDI	Unique device identifier		

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