DATA SHEET

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Clostridium perfringens Real Time PCR Kit
Cat. No.: DD-0167-02

For use with ABI Prism®7000/7300/7500/7900; Smart CyclerII;iCycler iQ™4/iQ™5; Rotor Gene™6000; Mx3000P/3005P;MJ-Option2/Chromo4;LightCycler®480real time PCR systems.

User Manual
For in vitro Diagnostic use only

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1. Intended Use
Clostridium perfringens real time PCR kit is used for the detection of Clostridium perfringens in stool or water samples by using real time PCR systems.

2. Principle of Real-Time PCR
The principle of the real-time detection is based on the fluorogenic 5'nuclease assay. During the PCR reaction, the DNA polymerase cleaves the probe at the 5' end and separates the reporter dye from the quencher dye only when the probe hybridizes to the target DNA. This cleavage results in the fluorescent signal generated by the cleaved reporter dye, which is monitored real-time by the PCR detection system. The PCR cycle at which an increase in the fluorescence signal is detected initially (Ct) is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities during Real Time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description
Clostridium perfringens (formerly known as C. welchii) is a Gram-positive, rod-shaped, anaerobic, spore-forming bacterium of the genus Clostridium. C. perfringens is ubiquitous in nature and can be found as a normal component of decaying vegetation, marine sediment, the intestinal tract of humans and other vertebrates, insects, and soil. C. perfringens is a human pathogen sometimes, and other times it can be ingested and not cause any harm.
Clostridium perfringens real time PCR kit contains a specific ready-to-use system for the detection of the Clostridium perfringens through polymerase chain reaction in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of the Clostridium perfringens DNA. Fluorescence is emitted and measured by the real time systems' optical unit during the PCR. The detection of amplified Clostridium perfringens DNA fragment is performed in fluorimeter **channel FAM** with the fluorescent quencher BHQ1. In addition, the kit contains a system to identify possible PCR inhibition by measuring the HEX/VIC/JOE fluorescence of the internal control (IC). An external positive control defined as 1×10^7copies/ml is supplied which allow the determination of the gene load. For further information, please refer to **section 9.3Quantitation.**

4. Kit Contents

Ref.	Type of Reagent	Presentation 25rxns
1	DNA Extraction Buffer	2 vials, 1.5ml
2	C. perfringens Reaction Mix	1 vial, 950µl
3	PCR Enzyme Mix	1 vial, 12µl
4	Molecular Grade Water	1 vial, 400µl
5	Internal Control	1 vial, 30µl
6	C. perfringens Positive Control(1×10^7copies/ml)	1 vial, 30µl

Analysis sensitivity: 1×10^3 copies/ml; LOQ: 2×10^3~1×10^8 copies/ml

5. Storage
- All reagents should be stored at -20°C. Storage at +4°C is not recommended.
- All reagents can be used until the expiration date indicated on the kit label.
- Repeated thawing and freezing (>3x) should be avoided, as this may reduce the sensitivity of the assay.
- Cool all reagents during the working steps.
- Reaction mix should be stored in the dark.

6. Additionally Required Materials and Devices
- Biological cabinet
- Vortex mixer
- Cryo-container
- Sterile filter tips for micro pipets
- Disposable gloves, powderless
- Refrigerator and Freezer
- Real time PCR system
- Real time PCR reaction tubes/plates
- Pipets (0.5µl – 1000µl)
- Sterile microtubes
- Biohazard waste container
- Tube racks
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)

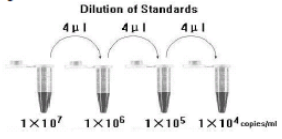
7. Warnings and Precaution
- Carefully read this instruction before starting the procedure.
- For in vitro diagnostic use only.
- This assay needs to be carried out by skilled personnel.
- Clinical samples should be regarded as potentially infectious materials and should be prepared in a laminar flow hood.
- This assay needs to be run according to Good Laboratory Practice.
- Do not use the kit after its expiration date.
- Avoid repeated thawing and freezing of the reagents, this may reduce the sensitivity of the test.
- Once the reagents have been thawed, vortex and centrifuge briefly the tubes before use.
- Quickly prepare the reaction mix on ice or in the cooling block.
- Set up two separate working areas: 1) Isolation of the RNA/ DNA and 2) Amplification/ detection of amplification products.
- Pipets, vials and other working materials should not circulate among working units.
- Use always sterile pipette tips with filters.
- Wear separate coats and gloves in each area.

8. Sample Collection, Storage and transportation
- Collect samples in sterile tubes;
- Specimens can be extracted immediately or frozen at -20°C to -80°C.
- Transportation of clinical specimens must comply with local regulations for the transport of etiologic agents

9. Procedure
9.1 Sampling and DNA extraction
9.1.1 Sampling and increasing bacteria
1) Sterilize the sample package and sampling tools before sampling.
2) Take 100g, 10g and 1g milk powder into three different culture bottles of 2L, 250ml and 125ml respectively.
3) Add 9 times volume sterilized water into these three culture bottles(M/V=1:9), and incubate them under 36±1℃for18~22h..
4) Take 10ml culture medium from the culture bottles into 90ml EE broth respectively, and incubate them for 18~22h.
9.1.2 DNA-Extraction
DNA extraction buffer is contained in the kit. Please thaw the buffer thoroughly and spin down briefly in the centrifuge before use.
9.1.1 Stool samples
1) Take about 50mg stool samples to a 1.5ml tube; add 1.0ml normal saline then vortex vigorously. Centrifuge the tube at 13000rpm for 2 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet.
2) Add 100µl DNA extraction buffer, close the tube then resuspend the pellet with vortex vigorously. Spin down briefly in a table centrifuge.
3) Incubate the tube for 10 minutes at 100°C.
4) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.
9.1.2 Water samples
1) Take 3 ml water to a tube, Centrifuge the tube at 13000rpm for 2 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet.
2) Add 100µl DNA extraction buffer, close the tube then resuspend the pellet with vortex vigorously. Spin down briefly in a table centrifuge.
3) Incubate the tube for 10 minutes at 100°C.
4) Centrifuge the tube at 13000rpm for 5 minutes. The supernatant contains the DNA extracted and can be used for PCR template.
Attention:
A. During the incubation, make sure the tube is not open, as the vapor will volatilize into the air and may cause contamination if the sample is positive.
B. The extraction sample should be used in 3 hours or store at -20°C for one month.
C. Different DNA extraction kits are available. You may use your own extraction systems or the commercial kit based on the yield. For the DNA extraction, please comply with the manufacturer's instructions.

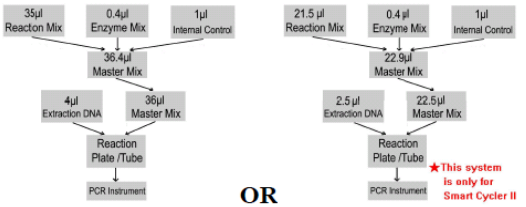
9.2 Internal Control and positive control
It is necessary to add internal control (IC) in the reaction mix. Internal Control (IC) allows the user to determine and control the possibility of PCR inhibition.
Add the internal control (IC) 1µl/rxn and the result will be got in the HEX/VIC/JOE channel.
Attention: It is necessary to dilute the internal control supplied in the kit by 10 times with molecular grade water before detection, and close the tube immediately then vortex for 10 seconds.
Because of transportation with carbon dioxide ice, there may be white precipitate in tubes of internal control and positive control ,but it will disappear in a few minutes when it is incubated at room temperature. Besides, the white precipitate have no effection on the detection result.

9.3 Quantitation
The kit can be used for **quantitative** or **qualitative** real-time PCR. A positive control defined as 1×10^7copies/ml is supplied in the kit.
For performance of quantitative real-time PCR, Standard dilutions must prepare first as follows.
Molecular Grade Water is used for dilution.
The step of dilution is not needed for performance of qualitative real-time PCR.
Take positive control (1×10^7copies/ml) as the starting high standard in the first tube. Respectively pipette **36ul** of Molecular Grade Water into next three tubes. Do three dilutions as the following figures:



To generate a standard curve on the real-time system, all four dilution standards should be used and defined as standard with specification of the corresponding concentrations.
Attention:
A. Mix thoroughly before next transfer.
B. The positive control (1×10^7copies/ml) contains high concentration of the target DNA.
Therefore, be careful during the dilution in order to avoid contamination.

9.4 PCR Protocol
The Master Mix volume for each reaction should be pipetted as follows:



※PCR system without HEX/VIC/JOE channel may be treated with 1µl Molecular Grade Water instead of 1µl IC.

1) The volumes of Reaction Mix and Enzyme Mix per reaction multiply with the number of samples, which includes the number of the controls, standards and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix the master mix completely then spin down briefly in a centrifuge.
2) Pipet **36µl (22.5µl for SmartCycer II)** Master Mix with micropipets of sterile filter tips to each *Real time* PCR reaction plate/tube. Then separately add **4µl (2.5µl for SmartCycer II)** DNA sample, positive and negative controls to different reaction plate/tubes. Immediately close the plate/tubes to avoid contamination.
3) Spin down briefly in order to collect the Master Mix in the bottom of the reaction tubes.
4) Perform the following protocol in the instrument:
37°C for 2 min, 1 cycle;94°C for 2 min, 1 cycle;93°C for 15 sec, 60°C for 60 sec, 40 cycles.
Fluorescence is measured at 60°C;FAM and HEX/VIC/JOE channels should be chosen.
5) If you use ABI Prism® system, please choose **"none"** as **passive reference** and **quencher.**
10. Baseline setting: just above the maximum level of molecular grade water.
11.Calabration for quantitative detection: Input each concentration of standard controls at the end of run, and a standard curve will be automatically formed.
12.Quality control: The Ct value of molecular grade water and positive control in FAM channel shows UNDET and ⩽35 respectively; The Ct value of internal control in HEX/VIC/JOE channel shows 25~33; Correlation coefficient of standard curve should be ⩽-0.98, otherwise the result is invalid.
13. Data Analysis and Interpretation
The following results are possible:
1) The Ct value in channel FAM shows ⩽35. **The result is positive: The sample contains Clostridium perfringens DNA.**
2) The Ct value in channel FAM shows 35~40, please repeat again. **If the result still shows 35~40,it can be considered negative.**
3) In channel FAM no signal is detected, at the same time, a HEX/VIC/JOE signal from the Internal Control appears. **The sample does not contain any Clostridium perfringens DNA. It can be considered negative.**
4) Neither in channel FAM nor in channel HEX/VIC/JOE signal is detected. **A diagnostic statement can not be made.** Inhibition of the PCR reaction.

For further questions or problems,please contact our technical support at **trade@liferiver.com.cn**

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