Liferiver™

Revision No.: ZJ0007
Issue Date: Jul 1st, 2012

Pseudomonas Aeruginosa Real Time PCR Kit
User Manual
For In Vitro Diagnostic Use Only

REF **QD-0203-01**

For use with LightCycler1.0/2.0 Instrument

EC REP Obelis S.A.
Boulevard Général Wahis 53
1030 Brussels, BELGIUM
Tel: +(32) 2.732.59.54
Fax: +(32) 2.732.60.03
E-Mail : mail@obelis.net

Shanghai ZJ Bio-Tech Co., Ltd.
www.liferiver.com.cn Tel: +86-21-34680596
trade@liferiver.com.cn Fax: +86-21-34680595
2nd floor,No.15 Building,No.188 Xinjunhuan Road,
PuJiang Hi-tech Park, Shanghai, China

1. Intended Use
Pseudomonas Aeruginosa real time PCR kit is used for the detection of Pseudomonas Aeruginosa by using real time PCR systems in samples like sputum, wound excretion, food, stool, urine, C.S.F, blood and etc.

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 is proportional to the amount of the specific PCR product. Monitoring the fluorescence intensities in real-time allows the detection of the accumulating product without having to re-open the reaction tube after the amplification.

3. Product Description
Pseudomonas aeruginosa is a Gram-negative, aerobic, rod-shaped bacterium with unipolar motility.An opportunistic human pathogen, P. aeruginosa is also an opportunistic pathogen of plants. P. aeruginosa is the type species of the genus Pseudomonas.Pseudomonas Aeruginosa real time PCR kit contains a specific ready-to-use system for the detection of the Pseudomonas Aeruginosa by polymerase chain reaction in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of the Pseudomonas Aeruginosa DNA. Fluorescence is emitted and measured by the real time systems´ optical unit. The detection of amplified Pseudomonas Aeruginosa DNA fragment is performed in fluorimeter **channel 530nm** DNA extraction buffer is available in the kit. In addition, the kit contains a system to identify possible PCR inhibition by measuring the 560nm fluorescence of the internal control (IC).

4. Kit Contents

Ref.	Type of Reagent	Presentation 25rxns
1	DNA Extraction Buffer	1 vial, 1.8ml
2	P. Aeruginosa Reaction Mix	1 vial, 450µl
3	PCR Enzyme Mix	1 vial, 12µl
4	Molecular Grade Water	1 vial, 400µl
5	Internal Control (IC)	1 vial, 30µl
6	P. Aeruginosa Positive Control (1×10^7copies/ml)	1 vial, 30µl

Analysis sensitivity: 1×10^4 copies/ml

Note: Analysis sensitivity depends on the sample volume, elution volume, nucleic acid extraction methods and other factors .If you use the DNA extraction buffer in the kit, the analysis sensitivity is the same as it declares. However, when the sample volume is dozens or even hundreds of times greater than elution volume by some concentrating method, it can be much higher.

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.
- Super 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.
- Prepare quickly 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.
- Do not pipette by mouth. Do not eat, drink, and smoke in laboratory.
- Avoid aerosols

8. Sample Collection, Storage and Transport
- Collected 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 DNA-Extraction
DNA extraction buffer is supplied in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use.

9.1.1 Sputum sample
1) **Trypsin digestive Solution preparation.** Add 10g trypsin to 200ml purified water and mix thoroughly. Adjust PH value to 8.0 with 2%NaOH solution. Add 2mL $CaCl_2$ (25mmol/L), mix thoroughly and store at 4℃. **Please incubate at 37℃ for 10 minutes before use.**

2) Estimate the volume of the sputum and add partes aequales of the Trypsin digestive Solution then vortex vigorously. Set at room temperature for 30 minutes. Transfer 0.5ml mixture to a new tube. Centrifuge the tube at 13000rpm for 5 minutes, carefully remove and discard supernatant from the tube without disturbing the pellet.
3) Add 1.0ml normal saline. Resuspend the pellet with vortex vigorously**.** Centrifuge at 13000rpm for 5 minutes. Carefully remove and discard supernatant from the tube without disturbing the pellet.
4) Repeat step 3)
5) Add 100µl DNA extraction buffer, close the tube then suspend the pellet with vortex vigorously**.** Spin down briefly in a table centrifuge.
6) Incubation the tube for 10 minutes at 100°C.
7) Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains DNA extracted and is used for PCR template.

9.1.2 Urine or C.S.F. sample
1) Take 1.5 ml sample 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 vortex for 10 seconds. 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.

9.1.3 Stool or food sample
1) Take about 30mg stool or 500mg food samples to a 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.

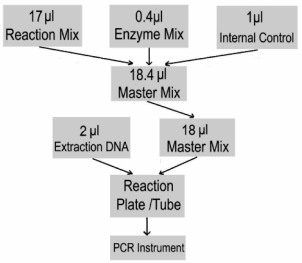
9.1.4 Wound excretion sample
1) Take 1ml sample in a tube, centrifuge the tube at 13000rpm for 2min, and remove the supernatant and keep the pellet.**2)** Add 100µl DNA extraction buffer to the pellet, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.**3)** Incubate the tube for 10 minutes at 100°C.**4)** Centrifuge the tube at 13000rpm for 10 minutes. The supernatant contains the DNA extracted and can be used for PCR template.

9.1.5 Blood sample
1) Take 2ml non-heparin anticoagulation, and transfer the plasma layer and buffy-coat layer to another tube after it is natural stratified.**2)** Add 100µl DNA extraction buffer into the tube, and lose the tube then vortex for 10 seconds. 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 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 in case the sample is positive.
B. The extraction sample should be used in 3 hours or stored at -20°C for one month.
C. DNA extraction kits are available from various manufacturers. You may use your own extraction systems or the commercial kit based on the yield. For DNA extraction, please comply with the manufacturer's instructions.

9.2 Internal 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 shown in the 560nm.

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



※PCR system without 560nm 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 controls and sample prepared. Molecular Grade Water is used as the negative control. For reasons of unprecise pipetting, always add an extra virtual sample. Mix completely then spin down briefly in a centrifuge.
2) Pipet **18µl Master** Mix with micropipets of sterile filter tips to each real time PCR reaction plate/tubes. Separately add **2µl** 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 2min	1cycle
94°C for 2min	1cycle
93°C for 5sec, 60°C for 30sec (Fluorescence measured at 60°C)	40cycles

Selection of fluorescence channels	
530nm	Target Nucleic Acid
560nm	IC

10. Threshold setting: Choose **Arithmetic** as back ground and **none** as Noise Band method, then adjust the Noise band just above the maximum level of molecular grade water, and adjust the threshold just under the minimum of the positive control.

11. Quality control: Negative control, positive control and internal control must be performed correctly, otherwise the sample results is invalid.

Channel / Control	Crossing point value	
	530nm	560nm
Molecular Grade Water	Blank	25~35
Positive Control(qualitative assay)	≤35	——

12. Data Analysis and Interpretation
The following results are possible:

	Crossing point value		Result Analysis
	530nm	560nm	
1#	Blank	25~35	Below the detection limit or negative
2#	≤35	——	Positive
3#	35～40	25~35	Re-test; If it is still 35~40, report as 1#
4#	Blank	Blank	PCR Inhibition; No diagnosis can be concluded.

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