1. Intended Use

Human parvovirus B19 real time PCR kit is used for the detection of human parvovirus B19 in serum or plasma 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

Parvovirus B19 was discovered by chance in 1975 at the Central Public Health Laboratory during routine screening for hepatitis B of asymptomatic blood donors from the South London Blood Transfusion Centre. B19 happened to be the serial number of the parvovirus positive specimen. Parvovirus B19 is a single-stranded DNA virus belonging to the Parvoviridae family of viruses, which includes a number of animal parvoviruses such as the canine parvovirus and feline panleukopenia virus. The virus is known to replicate in rapidly dividing erythroid progenitor cells. Other target cells are less well defined and may include myocardial tissue.

Human parvovirus B19 real time PCR kit contains a specific ready-to-use system for the detection of human parvovirus B19 (including genotype I, II and III) by polymerase chain reaction (PCR) in the real-time PCR system. The master contains reagents and enzymes for the specific amplification of human parvovirus B19 DNA. Fluorescence is emitted and measured by the real time systems´ optical unit during PCR. The detection of amplified B19 DNA fragment is performed in fluorimeter **channel 530nm with the fluorescent quencher BHQ1**. DNA extraction buffer is available in the kit and blood serum samples are used for DNA extraction. In addition, the kit contains a system to identify possible PCR inhibition by measuring the 560nm fluorescence of the internal control (IC). An external positive control (1×10^7copies/ml) allows the determination of the gene load. For further information, please refer to section 9.3 Quantitation.

4. Kit Contents

Ref.	Type of Reagent	Presentation 25rxns
1	DNA Extraction Buffer	1 vial, 1.8ml
2	B19 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	B19 Positive Control (1×10^7copies/ml)	1 vial, 30µl

Analysis sensitivity:5×10^3 copies/ml; LOQ: $1\times10^4\sim1\times10^8$ 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
- Real time PCR system
- Desktop microcentrifuge for "eppendorf" type tubes (RCF max. 16,000 x g)
- Vortex mixer
- Real time PCR reaction tubes/plates
- Cryo-container
- Pipets (0.5 µl – 1000 µl)
- Sterile filter tips for micro pipets
- Sterile microtubes
- Disposable gloves, powderless
- Biohazard waste container
- Refrigerator and freezer
- Tube racks

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, smoke in laboratory.
- Avoid aerosols

8. Sample Collection, Storage and transport

- 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 DNA-Extraction

DNA extraction buffer is supplied in the kit, please thaw the buffer thoroughly and spin down briefly in the centrifuge before use. You may use your own extraction systems or commercial kit**s.**

1) Pipet 50µl serum or plasma to a new 0.5ml tube, add 50µl DNA extraction buffer, close the tube then vortex for 10 seconds. Spin down briefly in a table centrifuge.

2) Incubate the tube for 10 minutes at 100°C.

3) Centrifuge the tube at 13000rpm for 10 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. Since 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 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 the 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.

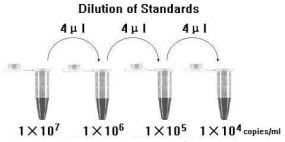
Add the internal control (IC) 1µl/rxn and the result will be shown in the 560nm.

9.3 Quantitation The kit can be used for **quantitative** or **qualitative** real-time PCR.

For performance of quantitative real-time PCR, Standard dilutions must prepare first as follows. Molecular Grade Water is used for dilution.

Dilution is not needed for performance of qualitative real-time PCR.

Take positive control (1×10 copies/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.

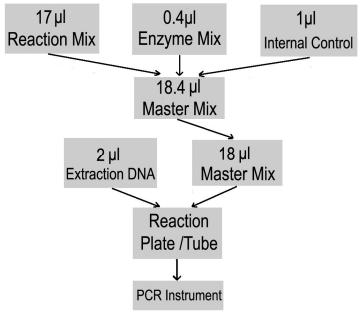
Attention:

A. Mix thoroughly before next transfer.

B. The positive control 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 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, 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 completely then spin down briefly in a centrifuge.

2) Pipet **18µl** Master Mix with micropipets of sterile filter tips to each of the *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.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: Negative control, positive control, internal control and QS curve must be performed correctly, otherwise the sample results is invalid.

Channel / Control	Crossing point value	
	530nm	560nm
Molecular Grade Water	Blank	25~33
Positive Control(qualitative assay)	≤35	——
QS（quantitative detection）	Correlation coefficient of QS curve≤－0.98	

13. Data Analysis and Interpretation

The following results are possible:

	Crossing point value		Result Analysis
	530nm	560nm	
1#	Blank	25~33	Below the detection limit or negative
2#	≤35	——	Positive; and the software displays the quantitative value
3#	35～40	25~33	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**