Scarlet™ Blood Direct PCR Kit (Heparin)

User's Instruction

Description

Scarlet™ Blood Direct PCR Kit (Heparin) is a research use only kit, which is ideal for direct PCR identification of Heparin anticoagulant whole blood without DNA purification. The 2 × Scarlet™ Blood Direct PCR Mix (Heparin) provided by this kit has strong inhibitor tolerance. The maximum volume of human blood sample as PCR template can reach 45% of PCR system and 20% for mouse blood. Scarlet™ Blood Direct PCR Kit (Heparin) can sensitively amplify genomic and exogenous DNA fragments in blood samples.

Kit Contents

	500 preps
1. 2 × Scarlet™ Blood Direct PCR Mix (Heparin)	1.7ml × 3
2. 6 × DNA Loading Buffer	1.5 ml

	2,000 preps
1. 2 × Scarlet™ Blood Direct PCR Mix (Heparin)	1.7ml × 12
2. 6 × DNA Loading Buffer	1.5 ml × 4

- 2 × Scarlet™ Blood Direct PCR Mix (Heparin): including modified Taq DNA polymerase, dNTPs, $MgCl_2$, buffer, enhancer, optimizer, and stabilizer. In PCR reaction, only the anticoagulant whole blood, primers and ddH_2O need to be added to the 2 × Scarlet™ Blood Direct PCR Mix (Heparin).
- 6 × DNA loading buffer: the loading buffer does not contain SDS. Please do not use other loading buffer containing SDS for the experiment, which will affect the result.

Features

- The whole blood is directly used as a PCR template, without time-consuming and expensive DNA purification and pretreatment.
- The system has strong amplification ability and can sensitively detect genomic and exogenous DNA fragments in the blood.
- The 2× Scarlet™ Blood Direct PCR Mix (Heparin) provided by this kit has strong inhibitor tolerance.

- The sample is operated in a fully enclosed condition, preventing either sample contamination or false positive PCR results.
- The Scarlet™ Blood Direct PCR Kit (Heparin) is specially designed for direct PCR identification of Heparin anticoagulant whole blood.

Limitations

- Amplified fragments should be less than 1 kb. If more than 1 kb, the amplification efficiency may decrease, or the amplification may even fail.
- If PCR products are used for sequencing, we recommend purifying the PCR products first.
- PCR products may have mutation.
- The 3' end of the PCR product is randomly added with A tail.

Protocol

Important Notes Before Starting:

1. Please use fresh anticoagulant samples for the experiment. If you only have frozen samples, please avoid repeated freeze-thaw, otherwise it will lead to smaller DNA fragments as PCR templates, affecting the efficiency of PCR negatively.
2. If the ambient temperature is high, 2 × Scarlet™ Blood Direct PCR Mix (Heparin) may become turbid. Please keep it on ice for 1-2 min, until the mix becomes clear, and then mix up and down for 3-5 times before use.

Setting Up Blood Direct PCR System

1. According to the table below, add 2 × Scarlet™ Blood Direct PCR Mix (Heparin), primers and ddH_2O into 200 μL PCR tube.
2. Add appropriate amount of anticoagulant blood to the above PCR system to dilute 2 × Scarlet™ Blood Direct PCR Mix (Heparin) to 1 x.
    a) Note: due to the differences in samples and storage time, we suggest finding the optimal volume of blood sample by exploring the concentration gradient conditions before large-scale PCR identification.

Component	Volume	Final concentration
2 × Scarlet™ Blood Direct PCR Mix (Heparin)	10 μl	1×
Forward Primer (10 μM)	0.5 μl	0.2-0.25 μM 1*
Reverse Primer (10 μM)	0.5 μl	0.2-0.25 μM 1*
Anticoagulated Blood 2*	X μl	
RNase-Free ddH_2O	(9-X) μl	-
Total Volume	20 μl	-

1*: Usually the ideal final concentration of primer is 0.2 μM. However, when the reaction performance is poor, the primer concentration can be adjusted in the range of 0.1-0.5 μM.

2*: If the template uses a collection card containing blood, blood with a diameter of 1-4 mm can be intercepted and directly added to a 20-50 μL PCR reaction system for amplification.

Thermocycling Conditions for Blood Direct PCR

Note: The PCR conditions below are for reference only. The optimal reaction conditions, including annealing temperature and extension time, need to be designed according to the template type, target fragment size, sequence of amplified fragments, GC content of primers, etc.

1. Initial denaturation: 94°C for 3 min
2. 35-40 cycles:
    a) 94°C for 10 sec
    b) 55-65°C for 20 sec (2 × Scarlet™ Blood Direct PCR Mix (Heparin) has good amplification capacity for templates with high GC content, so we recommend that the annealing temperature of all primers should be 2°C higher than the TM value when performing PCR).
    c) 72°C for 2kb/min (for DNA fragments less than 1kb, the recommended extension time is 30 sec).
3. Final extension: 72°C for 5 min
4. After the PCR reaction, transfer the final product to a centrifuge and centrifuge at

10,000 x g for 2 min. Collect the supernatant for electrophoresis.

a) Note: Due to the nature of blood, transparent gelatinous material will appear at the bottom of the PCR tube after the end of PCR reaction, which is normal.

5. Check the result by agarose gel electrophoresis.

a) Note: We recommend using 6 x DNA Loading Buffer in the kit. Please do not use loading buffer containing SDS for electrophoresis.

Transportation

Transport with ice bags to ensure that the kit is at <4°C.

Storage

If used frequently, 2 × Scarlet™ Blood Direct PCR Mix (Heparin) can be stored at 4°C for a short time (within 10 days). For long term storage, please keep it at - 20°C.

6 × DNA Loading Buffer can be stored at 4°C or - 20°C for a long time.