Blood Direct PCR Kit V2

PD103

Vazyme

Instruction for Use

Version 22.2

Vazyme

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01/Product Description

Blood Direct PCR Kit can perform PCR directly on whole blood samples without the need for DNA purification or sample pretreatment. This kit is applicable for fresh blood, blood stored at 4°C, frozen blood, and dried blood stains stored on Whatman903 or FTA commercial cards, and is compatible with all conventional anticoagulants (EDTA, Citrate Acid, Heparin, etc.). It contains Phanta Blood Super-Fidelity DNA Polymerase, which is modified based on Phanta Super-Fidelity DNA Polymerase. This enzyme has superior resistance to PCR inhibitors in whole blood samples and can amplify whole blood concentrations up to 40%. Equipped with optimized 2 × Phanta Blood Buffer V2, Blood Direct PCR Kit V2 can efficiently amplify genomic fragments up to 10 kb from whole blood samples.

Phanta Blood Super-Fidelity DNA Polymerase produces blunt-ended products, which are applicable to ClonExpress and TOPO cloning kits (Vazyme #C112/C113/C115/C601). This kit provides the positive control primer mix that is compatible with mammals and other vertebrates, which can be used to perform positive control reactions.

02/Components

Components	PD103-01 50 rxns (50 μl/rxn)	PD103-02 200 rxns (50 μl/rxn)
Phanta Blood Super-Fidelity DNA Polymerase (1 U/μl)	75 μl	300 μl
2 × Phanta Blood Buffer V2	1.25 ml	4 × 1.25 ml
Positive control primer mix (10 μM each)	50 μl	50 μl
10 mM each dNTPs	50 μl	200 μl
10 × DNA Loading buffer	1.25 ml	1.25 ml

03/Storage

Store at -30 ~ -15℃ and transport at ≤0℃.

04/Applications

It is applicable for PCR amplification of different types of whole blood samples.

05/Notes

For research use only. Not for use in diagnostic procedures.

1. The recommended usage of blood template is 1/10 of the total reaction volume, i.e., add 5 μl of whole blood into the 50 μl reaction system.
2. When sucking blood samples containing anticoagulants, especially those that have been stored for a long time, try to avoid sucking blood clots.

3. With whole blood as templates, the recommended final concentration of the enzyme is 1.5 U/50 μl reaction. Increasing the amount of enzyme can improve the amplification yield, but it should be ≤2 U/50 μl.
4. The extension time is set at 30 sec/kb. if less than 15 sec, set to 15 sec. For fragments ≥5 kb, if the amplification efficiency is low, it can be appropriately extended to 60 sec/kb.
5. It is recommended to centrifuge the reaction solution at 4,000 rpm (1,000 × g) for 1 - 3 min to precipitate the blood cell debris after the PCR amplification, and then take the supernatant for the downstream analysis.

Primer Desigh Guidance

1. It is recommended that the last base at the 3' end of the primer should be G or C;
2. Consecutive mismatches should be avoided in the last 8 bases at the 3' end of the primer;
3. Avoid hairpin structures at the 3' end of the primer;
4. The Tm value should be adjusted to 60 ~ 72℃ (Primer Premier 5 is recommended to calculate the Tm value);
5. Extra additional primer sequences that are not matched with the template, should not be included when calculating the primer Tm value;
6. It is recommended that the GC content of the primer to be 40% - 60%;
7. Tm value and GC content of forward and reverse primers should be as consistent as possible.

06/Experiment Process

1. Reaction System:

Mix all the components thoroughly after thawing. Please put them back to -20℃ immediately after use. Do not leave 2 × Phanta Blood Buffer V2 with lid open for a long time.

ddH_2O	To 50 μl
2 × Phanta Blood Buffer V2[a]	25 μl
10 mM each dNTPs	1 μl
Primer 1 (10 μM)[b]	2 μl
Primer 2 (10 μM)[b]	2 μl
Whole blood[c]	x μl
Phanta Blood Super-Fidelity DNA Polymerase (1 U/μl)[d]	1.5 μl

Please pipette up and down to mix thoroughly, then centrifuge it briefly to the bottom of the tube.

a. It contains Mg^{2+} at a final concentration of 2 mM.

b. The final concentration of each primer is recommended to be 0.4 μM. Too much primers may result in nonspecific amplification.

c. The optimal whole blood concentration range is 1% - 20%, and it is recommended to use 10% as initial test condition. Try to avoid sucking blood clots. If the sample is dried blood stains stored on Whatman filter paper card, about 1 mm^2 round paper with blood stains is recommended and placed directly into the reaction solution without pretreatment.

▲ Blood Direct PCR Kit V2 has been successfully tested on many mammalian species. In addition, whole blood samples of several birds have been successfully amplified with this kit. For birds and other species with nuclei in their blood cells, it may be necessary to reduce the amount of blood samples used for PCR amplification.

▲ For short-term storage (less than 3 months), whole blood can be stored at 4℃; For long-term storage, it is recommended to store at -20℃ or on Whatman FTA/930 card.

d. Phanta Blood Super-Fidelity DNA Polymerase is a high-fidelity polymerase with proofrea- ding activity, and its fidelity is 52 times higher than the *Taq* DNA polymerase. The enzyme has been mixed with monoclonal antibodies that can inhibit its exonuclease and polymerase activity at room temperature, and can perform highly specific hot-start PCR. With whole blood as templates, the recommended final concentration of the enzyme is 1.5 U/50 µl reaction. Increasing the amount of enzyme can improve the amplification yield, but it should be ≤2 U/50 µl.

▲ Phanta Blood Super-Fidelity DNA Polymerase has strong proofreading activity and the amplification product is blunt-ended. If TA cloning needs to be performed, it is recommended to purify the DNA before adding the adenine.

2. Reaction Program

Steps	Temperature	Time	Cycles
Initial Denaturation[a]	95℃	5 min	1
Denaturation	95℃	15 sec	35[d]
Annealing[b]	56 ~ 72℃	15 sec	
Extension[c]	72℃	30 sec/kb	
Final Extension	72℃	5 min	1

a. Initial denaturation (95℃, 5 min) can lyse white blood cells and release genomic DNA that can be used for PCR. Do not shorten the time or lower the temperature.

b. Phanta Blood Super-Fidelity DNA Polymerase can promote the primer anneal to the template with high efficiency. In general, please use an annealing temperature that equals to the Tm value of the primer. However, high annealing temperature can effectively reduce nonspecific amplification and improve the amplification efficiency of the whole blood template. Thus, if amplification products have low specificity, a temperature gradient reaction can be established to find the optimal annealing temperature of the primer. It is recommended to set the annealing time to 15 sec.

c. The extension time is set at 30 sec/kb. If less than 15 sec, set to 15 sec. For fragments ≥5 kb, if the amplification efficiency is low, it can be appropriately extended to 60 sec/kb.

d. In general, 35 cycles can amplify enough PCR products. Too many cycles will increase nonspecific amplification and decrease the fidelity.

3. Analysis of Amplification Product:

After PCR, it is recommended to centrifuge the reaction solution at 4,000 rpm (1,000 × g) for 1 - 3 min to precipitate the blood cell debris, and then take the supernatant for downstream analysis. This step can effectively remove a variety of blood components, especially when using high-concentration blood templates. The reason is that there will be a lot of blood cell debris in the reaction tube after PCR cycles. These debris can interfere with downstream assays, such as agarose electrophoresis assays. If the PCR product is required for Restriction Fragment Length Polymorphism (PCR-RFLP) analysis, it should be diluted 2 - 4 times in advance to remove the interference of salt and other inhibitors in the reaction solution.

4. Control Reactions:

This kit provides the positive control primer mix (10 μM each) for positive control reactions. The 237 bp fragment can be amplified from the genomes of mammals and many other vertebrates (Fig 1). The amplified region is located upstream of the sox21 gene, which is a highly conserved non-coding region.

Primer #1 (24-mer) 5' - AGCCCTTGGGGASTTGAATTGCTG -3' Tm: 69.5℃ (S=G or C);
Primer #2 (27-mer) 5' - GCACTCCAGAGGACAGCRGTGTCAATA -3' Tm: 67.9℃ (R=A), 71.5℃ (R=G). (Tm values are calculated with Primer Premier 5)

Reaction System

ddH_2O	15.5 μl
2 × Phanta Blood Buffer V2	25 μl
10 mM each dNTPs	1 μl
Positive control primer mix (10 μM each)	2 μl
Whole blood	5 μl
Phanta Blood Super-Fidelity DNA Polymerase (1 U/μl)	1.5 μl

Please pipette up and down to mix thoroughly, then centrifuge it briefly to the bottom of the tube.

Reaction Program

Steps	Temperature	Time	Cycles
Initial Denaturation	95℃	5 min	1
Denaturation	95℃	15 sec	35
Annealing	68℃	15 sec	
Extension	72℃	15 sec	
Final Extension	72℃	5 min	1

Fig 1. 237 bp fragments were amplified directly from whole blood of different species using the positive control primer mix. All blood samples were heparin anticoagulation stored at 4℃ and the concentration of blood template was 10%. NTC, no template control; PC (positive control) used purified human genomic DNA as amplification templates.

07/Examples

Take human whole blood (anticoagulation stored at 4℃) as a template to amplify target fragments with lengths of 237 bp, 492 bp, 501 bp, 1.0 kb, 1.1 kb, 1.5 kb, 2.0 kb, 2.5 kb, 3.8 kb, 7.5 kb, respectively. The amount of whole blood was 5 μl/50 μl reaction; the amount of Phanta Blood Super-Fidelity DNA Polymerase was 1.5 μl/50 μl reaction; the annealing temperature was 68℃; the extension time was 30 sec/kb (if less than 15 sec, set to 15 sec). As shown in Fig 2, all the fragments can be amplified efficiently.

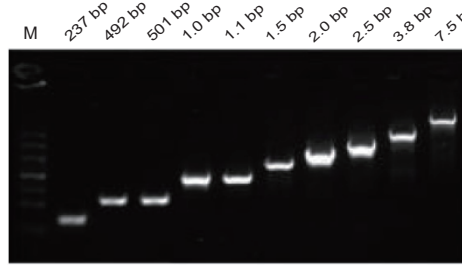

Fig 2. DNA fragments of different lengths were amplified directly from human whole blood using the Blood Direct PCR Kit. Blood samples were heparin anticoagulation stored at 4℃, and the concentration of blood template was 10%.

08/FAQ & Troubleshooting

◇No amplification product or low yield

① Check the design of the primers, verify the purity and concentration of primers
② Repeat the experiment to confirm the reaction system configuration and reaction procedure settings are correct
③ Increase the number of cycles
④ Increase the concentration of Mg^{2+}
⑤ Optimize the annealing temperature
⑥ Try different amount of blood

◇Products with high molecular weight smear bands

① Centrifuge and take the supernatant of the amplification products to do electrophoresis
② Extension time should not exceed 60 sec/kb
③ Optimize the annealing temperature
④ Try different amount of blood
⑤ Reduce the total number of cycles
⑥ Decrease primer concentration

◇Products with low molecular weight smear bands

① Centrifuge and take the supernatant of the amplification products to do electrophoresis
② Increase the annealing temperature
③ Try different amount of blood
④ Decrease primer concentration
⑤ Reduce the total number of cycles
⑥ Redesign primers

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