Vazyme

Vazyme biotech co., ltd.

ДИА•М современная лаборатория

www.dia-m.ru заказ on-line

Blood Direct PCR Kit V2

Catalog # PD103-01/02

Version 5.1

1. Introduction

Blood Direct PCR Kit is designed to perform PCR directly from whole blood without DNA extraction or sample preparation. This kit can be used to perform PCR amplification from a wide range of templates including fresh blood, 4℃ stored blood, frozen blood, and dried blood stored on Whatman 903® or FTA® commercial card. It is compatible with all conventional anticoagulants (EDTA, citrate, heparin, etc.).

This kit contains Phanta Blood Super-Fidelity DNA Polymerase, which is modified from Phanta Super-Fidelity DNA Polymerase. This enzyme displays superior resistance to the PCR inhibitors in the whole blood sample, and can amplify whole blood with concentration up to 40%. This kit can efficiently amplify genomic fragments of up to 10 kb from whole blood samples with the optimized 2 × Phanta Blood Buffer V2.

Phanta Blood Super-Fidelity DNA Polymerase produces blunt-ended products, which are applicable to ClonExpress cloning Kit (C112/C113). This kit supplies Positive control primer mix which is compatible with mammalian and other vertebrate animals and can be used as positive control in experiments.

2. Package Information

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

3. Storage

Store at -20 ℃.

4. Notes

-The recommended amount of blood as template is 1/10 of the total reaction volume, e.g. add 5 μl of the whole blood into a 50 μl reaction system.

- Be careful to avoid absorbing blood clots from anticoagulation, especially the anticoagulation stored for a long time.

- With whole blood as templates, the recommended amount of Phanta Blood Super-Fidelity DNA Polymerase is 1.5 U/50 μl for amplification. Increasing the amount of enzyme can improve the amount of PCR production. Please do not use enzyme with more than 2 U/50 μl.

- Extension time can be set as 30 sec/kb, at least 15 sec. If amplification efficiency is poor when amplify fragments > 5 kb, the extension time can be extended according to 60 sec/kb.

- It is recommended to centrifuge the reaction solution at 1,000 × g (about 4,000 rpm) for 1~3 min to deposit the blood cell debris after the PCR amplification, and then take the supernatant for the downstream analysis.

5. Protocol

1. General reaction mixture for PCR:

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

ddH_2O	13.5 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]	5 μl
Phanta Blood Super-Fidelity DNA Polymerase (1 U/μl) [d]	1.5 μl
Mix well by pipetting up and down.	

a. It contains 2 mM Mg^{2+}.

b. The recommended final concentration of each primer is 0.4 μM. Too much primer may result in non-specific amplification.

c. The optimal blood concentration ranges from 1% to 20%, and it is recommended to start with 10%. Be careful to avoid absorbing blood clots from anticoagulation. If the sample is dried blood on Whatman filter paper, please take about 1 mm^2 round papers with blood, and put it into the PCR reaction mixture directly without any pretreatment.

Note 1: Blood Direct PCR Kit V2 has been successfully tested with a number of mammalian species. In addition, whole blood samples of several poultry have been successfully amplified with this kit. For poultry and other species, of which the blood cells are with nuclear, the amount of blood used for the PCR reactions may be reduced.

Note 2: The whole blood can be stored at 4℃ for a short time (less than 3 months); for a long-term storage, it is recommended to store at -20℃ or on Whatman FTA® /903® cards.

d. Phanta Blood Super-Fidelity DNA Polymerase is a high fidelity polymerase with proofreading activity, and its fidelity is 52 times higher than the Taq DNA polymerase. The enzyme has been mixed with monoclonal antibodies which can inhibit its exonuclease and polymerase activity at room temperature, which make it applicable to highly specific hot start PCR. With whole blood as template, the recommended final concentration of the enzyme is 1.5 U per 50 µl reaction. Increasing the amount of enzyme can improve the amount of PCR production. Please do not use enzyme with more than 2 U/50 µl.

Note 1: Phanta Blood Super-Fidelity DNA Polymerase has strong proofreading activity, the amplification product was blunt-ended. The amplification products must be purified before adding A, if the following procedure is TA cloning.

2. Thermocycling program for a Routine PCR:

Cycling procedures	Temperature	Time	Cycle
Pre-denaturation [a]	95℃	5 min	1
Denaturation	95℃	15 sec	
Annealing [b]	56℃~ 72℃	15 sec	35 [d]
Extension [c]	72℃	30 sec/kb	
Complete extension	72℃	5 min	1

a. Pre-denaturation (95℃, 5 min) cracks leukocytes. The released genomic DNA can be used as template for PCR. Do not shorten the time or lower the temperature here.

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

c. Extension time can be set as 30 sec/kb, at least 15 sec. If amplification efficiency is poor when amplify fragments > 5 kb, the extension time can be extended according to 60 sec/kb.

d. Generally, 35 cycles are enough to amplify a sufficient amount of product. Too many cycles may result in non-specific amplification and low fidelity of amplification.

3. Amplification Product Analysis:

After amplification, centrifuge the PCR reaction mixture at 1 000 × g (about 4,000 rpm) for 1−3 minutes to deposit the blood cell debris after the PCR amplification, and then take the supernatant for the downstream analysis. This step removes various components of blood that might interfere with subsequent assays, e.g. gel electrophoresis. This step is especially important when high concentration of blood is used, as there can be a substantial amount of cell debris, etc. in the tube after the PCR reaction.

4. Control Reactions:

The kit provides positive control primer mix (10 µM each) for positive control reactions which amplify a 237-bp fragment of mammalian genomic DNA. The amplified region is a highly conserved noncoding region upstream of the *sox21* gene, and the primers are designed to amplify this region from a wide range of vertebrate species.

Primer #1 (24-mer) 5'- AGCCCTTGGGGASTTGAATTGCTG -3'

Tm: 69.5℃ (S=G or C), calculated with Primer Premier 5.

Primer #2 (27-mer) 5'- GCACTCCAGAGGACAGCRGTGTCAATA -3'

Tm: 67.9℃ (R=A), 71.5℃ (R=G), calculated with Primer Premier 5.

Reaction System

ddH_2O	15.5 µl
2 × Phanta Blood Buffer V2 [a]	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

Mix well by pipetting up and down

Reaction program

Cycling procedures	Temperature	Time	Cycle
Pre-denaturation	95℃	5 min	1
Denaturation	95℃	15 sec	
Annealing	68℃	15 sec	35
Extension	72℃	15 sec	
Complete extension	72℃	5 min	1

Figure 1. A 237-bp band can be amplified using Blood Direct PCR Kit V2 with whole blood of different species as templates and Positive control primer mix. The recommended concentration of blood template is 10%. All blood samples are heparin anticoagulation stored at 4℃.

NTC, no template control; PC, positive control, purified human genomic DNA as the amplification template.

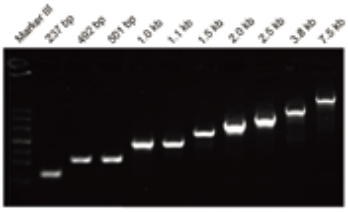

Figure 2. Amplify DNA fragments with different sizes directly from human whole blood using Blood Direct PCR Kit. Blood sample is heparin anticoagulation stored in 4℃, and the recommended working concentration of blood template is 10%.

5. Reaction Example:

Take human whole blood (anticoagulation stored at 4℃) as a template to amplify 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 target fragments, respectively. The recommended concentration of whole blood is 5 µl/50 µl reaction; The recommended concentration of Phanta Blood Super-Fidelity DNA Polymerase is 1.5 µl/50 µl reaction; The recommended annealing temperature is 68℃; The recommended extension time is 30 sec/kb (if less than 15 sec, please use 15 sec). As shown in Figure 2, all the fragments can be amplified efficiently.

7. Trouble shooting

No product at all or low yeild

- Check the design of the primers, verify the purity and concentration of primers.
- Repeat and make sure that the reaction mixture is well prepared and the PCR program is performed as recommended.
- Increase the number of cycles.
- Increased the concentration of Mg^{2+}.
- Optimize the annealing temperature.
- Try a different amount of blood.

Non-specific products - High molecular weight smears

- Centrifuge and use the supernatant of the amplification products to do electrophoresis.
- Extension time should not exceed 60 sec/kb.
- Optimize the annealing temperature.
- Try different amount of blood in the reaction.
- Reduce the total number of cycles.
- Decrease primer concentration.

Non-specific products - Low molecular weight discrete bands

- Centrifuge and use the supernatant of amplification products to do electrophoresis.
- Increase the annealing temperature.
- Try different amount of blood in the reaction.
- Decrease primer concentration.
- Reduce the total number of cycles.
- Design new primers.

8. Notes on whole blood amplification primer design

1. Choose C or G as the last base of the 3' end of primers;
2. Avoid continuous mismatch at the last 8 bases of the 3' end of the primer;
3. Avoid hairpin structure at the 3' end of the primer;
4. The Tm of primers should be 60℃ to 72℃, it is recommended to use the software Primer Premier 5 to determine the Tm values of primers;
5. The 5' added not matching sequence should not be included for the calculation of Tm of the primers;
6. The GC content of primer should be 40-60%;
7. The Tm value and GC content of forward primer should be as consistent with the reverse prime as possible.

ООО «Диаэм»
Москва
ул. Магаданская, д. 7, к. 3 ■ тел./факс: (495) 745-0508 ■ sales@dia-m.ru
www.dia-m.ru

С.-Петербург
+7 (812) 372-6040
spb@dia-m.ru

Новосибирск
+7(383) 328-0048
nsk@dia-m.ru

Воронеж
+7 (473) 232-4412
vrn@dia-m.ru

Йошкар-Ола
+7 (927) 880-3676
nba@dia-m.ru

Красноярск
+7(923) 303-0152
krsk@dia-m.ru

Казань
+7(843) 210-2080
kazan@dia-m.ru

Ростов-на-Дону
+7 (863) 303-5500
rnd@dia-m.ru

Екатеринбург
+7 (912) 658-7606
ekb@dia-m.ru

Кемерово
+7 (923) 158-6753
kemerovo@dia-m.ruu

Армения
+7 (094) 01-0173
armenia@dia-m.ru