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PRODUCT INFORMATION

Thermo Scientific
Phusion Blood Direct PCR Master Mix

Pub. No. MAN0012900
Rev. Date 05 December 2016 (Rev. B.00)

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Lot: __ Expiry Date: __

Store at -20 °C

Ordering information

Component	#F-175S 100 rxns	#F-175L 500 rxns
2X Phusion Blood Direct PCR Master Mix	1 mL	5 × 1 mL
50 mM EDTA (pH 8.0)	0.5 mL	0.5 mL
50 mM $MgCl_2$ solution	1.5 mL	1.5 mL
100 % DMSO	0.5 mL	0.5 mL
Universal control primer mix	40 µL	40 µL
Water, nuclease- free	1.25 mL	4 × 1.25 mL
O'GeneRuler Express DNA Ladder	100 appl. (50 µg)	

Storage

Upon arrival, store the components at -20 °C.

www.thermofisher.com

1. Introduction

Thermo Scientific™ Phusion™ Blood Direct Master Mix is designed to perform PCR directly from whole blood with no prior DNA extraction or sample preparation. Blood stored at 4 °C or frozen, and preserved with EDTA, citrate or heparin are all suitable for this Master Mix, as is blood dried onto commercially available cards such as Whatman™ 903 and FTA™ Cards. Both genomic and exogenous target DNA can be amplified.

The Phusion Blood Direct PCR Master Mix employs a modified Thermo Scientific™ Phusion™ Hot Start II High-Fidelity DNA Polymerase that exhibits extremely high resistance to inhibitors found in blood. The Master Mix has been optimized to give excellent results from mammalian blood with amplicons up to 7.5 kb. The recommended blood concentration is 1−20 %, although robust amplification with up to 40 % blood can often be achieved with some optimization. This Master Mix is recommended for end-point PCR protocols and it contains premixed gel loading dye which allows direct sample loading on the gel. The loading dye in the Master Mix does not interfere with PCR performance and is compatible with downstream applications such as DNA sequencing, ligation and restriction digestion.

The Phusion Blood Direct PCR Master Mix includes a pair of universal control primers that are compatible with a number of mammalian and other vertebrate species (see Section 7).

2. Important Notes

- The annealing rules are different from many common DNA polymerases (such as *Taq* DNA polymerases). Read Section 5.2 carefully.
- The recommended starting amount is 5 % blood added directly to the reaction without further modification.
- After PCR, spin the reactions at 1 000 × g (about 4 000 rpm) for 1−3 minutes to pellet debris from blood.
- Phusion DNA Polymerases produce blunt end PCR products.

3. Guidelines for PCR

Carefully mix and spin down all tubes before opening to ensure homogeneity and improve recovery. The PCR setup can be performed at room temperature.

Table 1. Pipetting instructions

Component	20 µL rxn	50 µL rxn	Final conc.
2X Phusion Blood Direct PCR Master Mix	10 µL	25 µL	1X
Primer A	X µL	X µL	0.5 µM
Primer B	X µL	X µL	0.5 µM
Whole Blood	1 µL	2.5 µL	(See 4.1)
H_2O	Add to 20 µL	add to 50 µL	
Optional components for reactions optimization			
50 mM $MgCl_2$	0.6 µL	1.5 µL	(See 4.3)
50 mM EDTA	0.5-1.0 µL	1.25-2.5 µL	(See 4.3)
DMSO	1.0 µL	2.5L	5 % (See 4.4)

Table 2. Recommended cycling protocol

Cycle step	2-step protocol		3-step protocol		Cycles
	Temp.	Time	Temp.	Time	
Lysis of cells (see 5.1)	98 °C	5 min	98 °C	5 min	1
Denaturation	98 °C	1 s	98 °C	1 s	35-40
Annealing (see 5.2)	-	-	X °C	5 s	
Extension (see 5.3)	72 °C	15-30 s/kb	72 °C	15-30 s/kb	
Final Extension	72 °C 4 °C	1 min hold	72 °C 4 °C	1 min hold	1

Gel electrophoresis

2X Phusion Blood Direct PCR Master Mix contains a premixed gel loading dye. After PCR samples can be directly loaded on the electrophoresis gel for analysis.

Positive control reaction with purified DNA

When optimizing the reactions, it is recommended to perform a positive control with purified DNA to ensure that the PCR conditions are optimal. If the positive control with purified DNA fails, the PCR conditions should be optimized before continuing further.

Negative control

It is recommended to add a no-template control to all Direct PCR assays to control contamination.

4. Notes about reaction components

4.1. Blood sample

With the Phusion Blood Direct PCR Master Mix, it is possible to use a wide range of blood concentrations in the reaction (from 1% to 20%, or in some cases even up to 40%). The recommended starting point is 5%.

In general, if higher blood percentage (>10%) is used, higher reaction volume (up to 50 µL) is recommended, and some optimization may be required (in particular the $MgCl_2$ concentration may need to be increased up to 4.5 mM final concentration). For small amplicons, whole blood up to 40% can be used. More information about blood volumes for specific species available at **www.thermofisher.com.**

If blood dried onto a Whatman card is used, a 1 mm punch can be added directly to the reaction without pretreatment. The optimal reaction volume varies depending on the card type used:

- Whatman 903 Card: 10−50 µL
- Whatman FTA Elute Card: 25−50 µL
- Whatman FTA Gene Card: 50 µL

If larger punches or smaller volumes are desired for the FTA cards, the punches may need to be pretreated by washing with 20 µL H_2O at 50 °C for 3 minutes. After washing, the water can simply be removed and the PCR mix added directly to the rinsed punch.

Non-mammalian blood

The Phusion Blood Direct PCR Master Mix has been successfully tested with a variety of mammalian species. In addition, good results have been obtained with several different bird species. For birds and other species with nucleated blood cells, it may be necessary to reduce the amount of blood in the PCR reaction.

Blood storage

For long term storage, it is recommended to store blood at −20 °C or dried on Whatman FTA/903 Cards. For short term storage (less than 3 months), blood can be stored at 4 °C. EDTA, sodium citrate and heparin all work well with the Phusion Blood Direct PCR Master Mix.

4.2. Enzyme

Phusion Blood II DNA Polymerase is a proofreading polymerase that contains a unique processivity-enhancing domain, making this polymerase accurate and rapid. Phusion Blood II DNA Polymerase is a hot start polymerase utilizing a reversibly binding Affibody™ protein[1,2]. Phusion Blood II DNA Polymerase possesses 5'→3' DNA polymerase activity and 3'→5' exonuclease activity. The error rate of Phusion Blood II DNA Polymerase is approximately 25-fold lower than that of *Thermus aquaticus* DNA polymerase when determined with a modified *lac*I-based method[3].

The recommended amount of Phusion Blood II DNA Polymerase is optimized to work with most amplicons. When cloning fragments amplified with Phusion Blood II DNA Polymerase, blunt end cloning is recommended. If TA cloning is required, it can be performed by adding A overhangs to the blunt PCR product with *Taq* DNA Polymerase, for example. However, before adding the overhangs it is very important to remove all Phusion Blood II DNA Polymerase by purifying the PCR product carefully. Any remaining Phusion Blood II DNA Polymerase will degrade the A overhangs, creating blunt ends again. A detailed protocol for TA cloning of PCR fragments amplified with any of the Phusion DNA Polymerases can be found on our website: www.thermofisher.com.

4.3. $MgCl_2$ concentration

The 2X Phusion Blood PCR Master Mix included in the kit provides 3.0 mM $MgCl_2$ in the final reaction. Extensive testing has shown 3.0 mM $MgCl_2$ to be optimal for most PCR reactions using whole blood. There is a separate tube of 50 mM $MgCl_2$ (F-510MG) included in the kit for adjusting the $MgCl_2$ concentration (up to 4.5 mM if necessary) for reactions containing very high amounts of blood. It should be noted, though, that excess $MgCl_2$ may result in spurious PCR products. If unspecific products are created with the default reaction buffer (providing 3.0 mM $MgCl_2$), the effective $MgCl_2$ concentration can be decreased by adding the chelating agent EDTA, which is included in the kit. Typically, adding 0.5 to 1.0 µL of 50 mM EDTA to a 20 µL reaction is sufficient to eliminate non-specific products. Note that the optimal conditions will depend on the primers, the percentage of blood in the reaction and/or the type of card used, since anticoagulants and other chemicals impregnating the cards can alter the available Mg^{2+} concentration.

4.4. DMSO

DMSO has been found to improve PCR results for some amplicons (particularly GC-rich templates). With these amplicons it is recommended to add 1−5% DMSO to the reaction. Note that if high DMSO concentration is used, the annealing temperature must be decreased, as DMSO alters the melting point of the primers. It has been reported that 10% DMSO decreases the annealing temperature by 5.5−6.0 °C[4].

Other PCR additives such as formamide, glycerol, and betaine are also compatible with Phusion Blood II DNA Polymerase.

5. Notes about cycling conditions

5.1. Lysis of cells and DNA denaturation

The initial 5-minute incubation step at 98 °C allows the lysis of leukocytes, making genomic DNA available for PCR. The DNA denaturation step can be very short. It is sufficient that the reaction mixture reaches the required 98 °C.

5.2. Primer annealing

Note that the optimal annealing temperature for Phusion Blood II DNA Polymerase may differ significantly from that of *Taq*-based polymerases. Always use the Tm calculator and instructions on our website (www.thermofisher.com/tmcalculator) to determine the Tm values of primers and optimal annealing temperature. As a basic rule, for primers >20 nt, anneal for 5 seconds at a Tm +3 °C of the lower Tm primer. For primers ≤20 nt, use an annealing temperature equal to the Tm of the lower Tm primer. If necessary, use a temperature gradient to find the optimal annealing temperature for each template-primer pair combination. The annealing gradient should extend up to the extension temperature (two-step PCR). Two-step cycling without an annealing step is recommended for high-Tm primer pairs (Tm at least 69−72 °C).

5.3. Extension

The extension is performed at 72 °C. The optimal extension time varies depending on the amplicon length and the blood concentration in the reaction. The recommended extension time is 15 seconds for amplicons ≤1 kb, and 30 s/kb for amplicons >1 kb. When higher blood concentrations are used, longer extension times may improve the results.

6. Post-amplification analysis

After amplification, centrifuge the PCR reaction at 1 000 × g for 1−3 minutes to collect the supernatant for analysis. This step separates the various components of blood that might interfere with subsequent assays, e.g. gel electrophoresis. This is especially important when high blood concentrations are used, as there can be a substantial amount of cell debris, etc. in the tube after the PCR reaction.
If post-PCR enzyme treatment is performed (e.g. PCR-RFLP), it may be necessary to dilute the PCR product 2−4-fold in order to dilute the salts and other inhibitors from the PCR reaction.

7. Control reactions

7.1. Universal control primer mix

This component is a mix of degenerate primers in H_2O that amplify a 237 bp fragment of mammalian genomic DNA. The amplified region is a highly conserved non-coding region upstream of the SOX21 gene[5], and the primers are designed to amplify this region from a wide range of vertebrate species. Each primer concentration is 25 µM.
Primer #1 (24-mer)
5'- AGCCCTTGGGGASTTGAATTGCTG -3'
Melting point: 73.5 °C

Primer #2 (27-mer)
5'- GCACTCCAGAGGACAGCRGTGTCAATA -3'
Melting point: 72.2°C (R=A), 75.3 °C (R=G)

Table 3. Pipetting instructions for control reactions

Component	20 µL rxn	50 µL rxn
H_2O	Add to 20 µL	add to 50 µL
2X Phusion Blood Direct PCR Master Mix	10 µL	25 µL
Universal Control Primer Mix	0.4 µL	1 µL
Whole Blood (see 4.1)	Up to 1 µL	Up to 10 µL

Table 4. Cycling instructions for control reactions

Cycle step	Temp.	Time	Cycles
Lysis of cells	98 °C	5 min	1
Denaturation Annealing/Extension	98 °C 72 °C	1 s 15 s	35
Final Extension	72 °C 4 °C	1 min hold	1

8. Troubleshooting

No product at all or low yield	
• Repeat and make sure that there are no pipetting errors. • Make sure that the cycling protocol was performed as recommended. • Optimize annealing temperature. • Titrate the amount of blood in the reaction. • Increase the number of cycles. • Check the purity and concentration of the primers.	• Check primer design. • Increase extension time. • Increase denaturation time up to 5 seconds. • Add 1−5 % DMSO (especially for high GC amplicons). • Add $MgCl_2$ (1−2 µL/50 µL reaction) if high blood concentration is used. • Add EDTA (1.25−2.5 µL/50 mL reaction).
Non-specific products - High molecular weight smears	
• Make sure that the extension time used was not too long. (Recommended extension time is 15−30 s/kb). • Increase annealing temperature or perform a temperature gradient PCR.	• Titrate the amount of blood in the reaction. • Reduce the total number of cycles. • Decrease primer concentration. • Add EDTA (1.25−2.5 µL/50 µL reaction).
Non-specific products - Low molecular weight discrete bands	
• Increase annealing temperature. • Titrate the amount of blood in the reaction. • Shorten extension time.	• Perform a temperature gradient PCR. • Decrease primer concentration. • Add EDTA (1.25−2.5 µL/50 mL reaction). • Design new primers.

CERTIFICATE OF ANALYSIS

Performance in PCR is tested by the amplification of 7.5 kb fragment from human genomic DNA.
Absorption measured at 424 nm and 614 nm.

Quality authorized by: Jurgita Zilinskiene

REFERENCES

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