Direct PCR from blood using Platinum SuperFi DNA Polymerase

Introduction

Invitrogen™ Platinum™ SuperFi™ DNA Polymerase is engineered with a DNA-binding domain resulting in high processivity and increased resistance to PCR inhibitors present in blood, as well as to most widely used blood preservatives (e.g., heparin, EDTA, citrate). This application note describes how researchers can successfully perform PCR from blood samples without DNA purification using Platinum SuperFi DNA Polymerase. Multiplex PCR can also be used to amplify more than one target sequence directly from blood. This protocol minimizes sample loss and helps significantly reduce protocol time with no DNA purification or isolation steps.

Materials and methods

- Whole blood
- Invitrogen™ Platinum™ SuperFi™ PCR Master Mix (Cat. No. 12358010) or Platinum SuperFi DNA Polymerase (Cat. No. 12351010)
- Invitrogen™ dNTP Mix (10 mM each, Cat. No. 18427013; only required for stand-alone enzyme)
- Forward and reverse primers
- 50 mM $MgCl_2$
- Invitrogen™ E-Gel™ General Purpose Agarose Gels or equivalent

Important notes

- Annealing temperature rules for Platinum SuperFi DNA Polymerase are different from many common DNA polymerases (such as *Taq* DNA polymerases). For optimal results, use the T_m calculator on our website at **thermofisher.com/tmcalculator**
- Platinum SuperFi DNA Polymerase may be used for amplification of DNA from a wide range of blood concentrations in the reaction. The recommended starting amount is 5% blood added directly to the reaction without further modification. Extensive mixing or vortexing after addition of blood to the PCR master mix should be avoided.
- $MgCl_2$ concentration may need to be increased up to 4.5 mM final concentration, especially if a higher blood percentage (≥10%) is used.
- After PCR, centrifuge the reactions at 1,000 × *g* (about 4,000 rpm) for 1–3 minutes to pellet debris from blood.

Below are recommendations for the amount of blood that is needed for direct PCR. These recommendations can be used as guidelines when working with a new sample. Reaction setup and thermal cycling protocols are also provided.

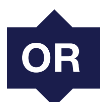
Recommended blood amount for direct PCR

Sample	Concentration (v/v)	Volume in 20 µL reaction	Volume in 50 µL reaction
Human blood (preserved with EDTA, heparin, or citrate)	1–20%	0.2–4 µL	0.5–10 µL

Reaction conditions for PCR using master mix

Component	20 µL reaction	50 µL reaction	Final concentration
Water, nuclease-free	to 20 µL	to 50 µL	–
2X Platinum SuperFi PCR Master Mix*	10 µL	25 µL	1X
10 µM forward primer	1 µL	2.5 µL	0.5 µM each
10 µM reverse primer	1 µL	2.5 µL	0.5 µM each
Whole blood	0.2–4 µL	0.5–10 µL	1–20%
Optional components for reaction optimization			
50 mM $MgCl_2$	0.6–1.2 µL	1.5–3 µL	Up to 4.5 mM
5X SuperFi™ GC Enhancer**	4 µL	10 µL	1X

* Provides 1.5 mM $MgCl_2$ in final reaction concentration.
**Recommended for targets with >65% GC content.



Reaction conditions for PCR using stand-alone enzyme

Component	20 µL reaction	50 µL reaction	Final concentration
Water, nuclease-free	to 20 µL	to 50 µL	–
5X SuperFi™ Buffer*	4 µL	10 µL	1X
10 mM dNTP mix	0.4 µL	1 µL	0.2 mM each
10 µM forward primer	1 µL	2.5 µL	0.5 µM each
10 µM reverse primer	1 µL	2.5 µL	0.5 µM each
Whole blood	0.2–4 µL	0.5–10 µL	1–20%
Platinum SuperFi DNA Polymerase (2 U/µL)	0.2 µL	0.5 µL	0.02 U/µL
Optional components for reaction optimization			
50 mM $MgCl_2$	0.6–1.2 µL	1.5–3 µL	Up to 4.5 mM
5X SuperFi GC Enhancer**	4 µL	10 µL	1X

* Provides 1.5 mM $MgCl_2$ in final reaction concentration.
**Recommended for targets with >65% GC content..

Cycling protocol

PCR cycles	Step	2-step protocol		3-step protocol	
		Temp.	Time	Temp.	Time
1	Initial denaturation*	98°C	30 sec	98°C	30 sec
30–35	Denature	98°C	5–10 sec	98°C	5–10 sec
	Anneal**	–	–	Varies	10 sec
	Extend	72°C	30 sec/kb	72°C	30 sec/kb
1	Final extension	72°C	5 min	72°C	5 min
		4°C	Indefinitely	4°C	Indefinitely

* The initial denaturation step can be extended for up to 5 minutes to ensure complete cell lysis; however, 30 sec is usually sufficient.

** Always use the T_m calculator on our website at **thermofisher.com/tmcalculator** to calculate the T_m of your primers and the recommended annealing temperature.

After amplification, centrifuge the PCR reaction at 1,000 x 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). Centrifugation is especially important when high blood concentrations are used, as there can be a substantial amount of cell debris in the tube after the PCR reaction. The sample should be diluted 2- to 20-fold for optimal separation using E-Gel agarose gels.

Results

A 585 bp fragment was amplified from whole blood using Platinum SuperFi PCR Master Mix. Varying percentages of human blood (from 1% to 20% (v/v)) preserved with EDTA, heparin, or citrate were added directly to the PCR reactions. $MgCl_2$ concentration was increased to 3.5 mM final concentration by adding 50 mM $MgCl_2$ solution. The cycling protocol was: 1 cycle at 98°C for 30 sec; 35 cycles at 98°C for 10 sec, 65°C for 10 sec, and 72°C for 20 sec; 1 cycle at 72°C for 5 min. The products were separated using 1% agarose gels in TAE buffer. Successful amplification was achieved with up to 20% EDTA- or heparin-stabilized blood or up to 10% citrate-stabilized blood (Figure 1).

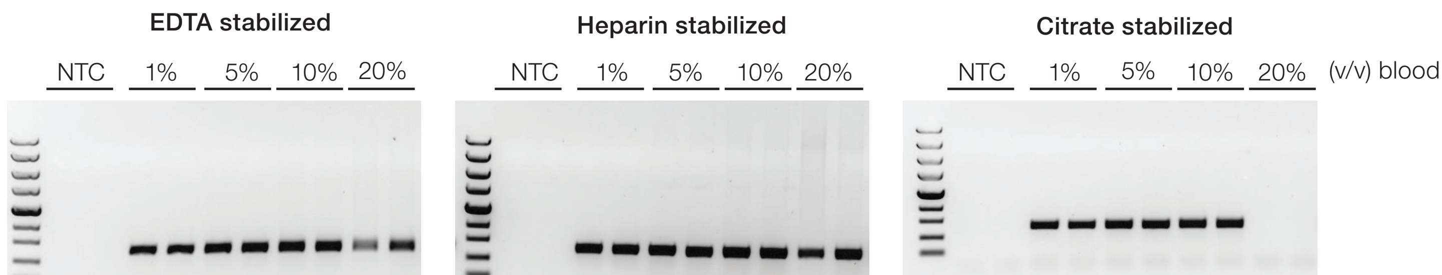


Figure 1. Direct amplification from whole blood. Amplification of a 585 bp fragment from varying percentages of human blood (v/v) was performed using Platinum SuperFi PCR Master Mix. $MgCl_2$ was added to 3.5 mM final concentration. Thermo Scientific™ ZipRuler™ Express DNA Ladder 2 was used as a size standard. NTC: no template control.

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To demonstrate the ability to perform multiplex PCR, we simultaneously amplified 8 targets from whole human blood (Figure 2). PCR reactions contained 0.3 µM of each primer and 1 µL of whole blood per 50 µL of PCR reaction. The cycling protocol was: 1 cycle at 98°C for 30 sec; 30 cycles at 98°C for 10 sec, 65°C for 30 sec (30 sec annealing is recommended for multiplex PCR), and 72°C for 15 sec; 1 cycle at 72°C for 5 min. The products were separated using a 2% agarose gel in TAE buffer.

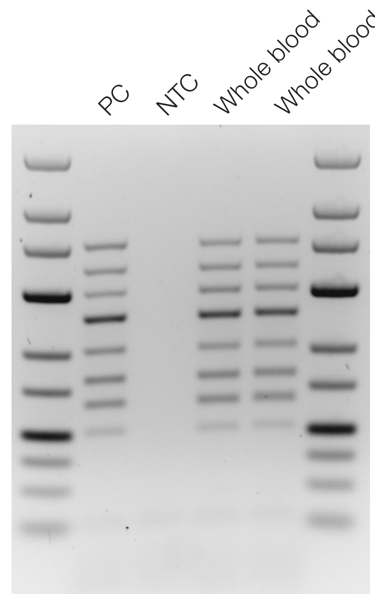

Figure 2. Multiplex PCR from human blood using Platinum SuperFi DNA Polymerase. Eight targets (99, 131, 160, 199, 251, 300, 345, and 400 bp) were amplified in the same 50 µL PCR reaction from 1 µL of whole blood. Thermo Scientific™ O'GeneRuler™ Low Range DNA Ladder was used as a size standard. NTC: no template control; PC: positive control from 10 ng of purified human genomic DNA.

Summary

With its high processivity and resistance to inhibitors, Platinum SuperFi DNA Polymerase enables amplification of DNA directly from blood as well as successful multiplex PCR from whole blood. These procedures can help streamline PCR workflows for multiple applications involving blood samples.

Find out more at **thermofisher.com/platinumsuperfi**

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