Direct PCR from blood using Platinum II *Taq* Hot-Start DNA Polymerase

Introduction

Invitrogen™ Platinum™ II *Taq* Hot-Start DNA Polymerase combines speed and inhibitor tolerance with an innovative buffer that allows one universal temperature for primer annealing. Platinum II *Taq* Hot-Start DNA Polymerase shows increased tolerance to substances originating from blood as well as to most widely used blood preservatives (e.g., EDTA, citrate). This allows PCR amplification from blood samples without a DNA isolation step. Here we provide recommendations for fast and convenient PCR using Platinum II *Taq* DNA polymerase for multiple applications involving blood samples.

Important notes

- Due to the unique composition of the Platinum II PCR buffer, the annealing temperature is 60°C for any primer pair designed following the general design rules. Isostabilizing molecules in the reaction buffer increase primer–template duplex stability during the annealing step and contribute to enhanced specificity without the need to optimize the annealing temperature for each primer pair.
- Platinum II *Taq* Hot-Start DNA Polymerase can amplify DNA from a wide range of blood concentrations. The recommended starting amount is 5% blood added directly to the reaction without further modification. Avoid extensive mixing or vortexing after addition of blood to the PCR mix.
- If amplification does not give satisfactory results, the final concentration of $MgCl_2$ may need to be increased, up to 4.5 mM.
- After PCR, centrifuge the reactions at 1,000 x *g* (about 4,000 rpm) for 1–3 minutes to pellet debris from the blood.

Materials and methods

- Whole blood
- Invitrogen™ Platinum™ II Hot-Start PCR Master Mix (2X) (Cat. No. 14000012) or Platinum II *Taq* Hot-Start DNA Polymerase (Cat. No. 14966001)
- Invitrogen™ dNTP Mix (10 mM each, Cat. No. 18427013; only required for stand-alone enzyme)
- Forward and reverse primers
- 25 mM $MgCl_2$
- Invitrogen™ E-Gel™ General Purpose Agarose Gels or equivalent

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When working with new PCR conditions, we recommend starting with the guidelines below for reaction setup and thermal cycling (Tables 1–3). Platinum II *Taq* Hot-Start DNA Polymerase allows for flexibility in reaction setup, while Platinum II Hot-Start PCR Master Mix (2X) provides more convenience for researchers since it contains all the necessary reaction components except primers and template DNA, therefore reducing setup time and pipetting steps. Additional recommendations for reaction optimization are provided.

Table 1. Reaction conditions for PCR using master mix.

Component	20 µL reaction	50 µL reaction	Final concentration
Platinum II Hot-Start PCR Master Mix (2X)*	10 µL	25 µL	1X
10 µM forward primer	0.4 µL	1 µL	0.2 µM each
10 µM reverse primer	0.4 µL	1 µL	0.2 µM each
Human blood	0.2–4 µL	0.5–10 µL	1–20%
Water, nuclease-free	To 20 µL	To 50 µL	–
Optional components for reaction optimization			
25 mM $MgCl_2$	1.2–2.4 µL	3–6 µL	Up to 4.5 mM
Platinum GC Enhancer**	4 µL	10 µL	1X

* Provides 1.5 mM $MgCl_2$ in final reaction.

** Recommended for targets with >65% GC content.

Table 2. Reaction conditions for PCR using stand-alone enzyme.

Component	20 µL reaction	50 µL reaction	Final concentration
5X Platinum II PCR Buffer*	4 µL	10 µL	1X
10 mM dNTP Mix	0.4 µL	1 µL	0.2 mM each
10 µM forward primer	0.4 µL	1 µL	0.2 µM each
10 µM reverse primer	0.4 µL	1 µL	0.2 µM each
Human blood	0.2–4 µL	0.5–10 µL	1–20%
Platinum II *Taq* Hot-Start DNA Polymerase	0.16 µL	0.4 µL	0.04 U/µL
Water, nuclease-free	To 20 µL	To 50 µL	–
Optional components for reaction optimization			
25 mM $MgCl_2$	1.2–2.4 µL	3–6 µL	Up to 4.5 mM
Platinum GC Enhancer**	4 µL	10 µL	1X

* Provides 1.5 mM $MgCl_2$ in final reaction.

** Recommended for targets with >65% GC content.

Table 3. Cycling protocol.

PCR cycles	Step	Temperature (°C)	Time
1	Initial denaturation	94	2 min
30–35	Denaturation	94	15 sec
	Annealing*	60	15 sec
	Extension	68	15 sec/kb
Hold		4	Indefinitely

* An annealing temperature of 60°C works for most primers. There may be cases when additional optimization of the annealing temperature will be desirable.

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Often debris of various blood components is formed in the reaction mix after PCR cycling, especially if high blood concentrations were used. To separate the debris, centrifuge the PCR reaction at 1,000 x *g* for 1–3 minutes and collect the supernatant for further analysis (e.g., gel electrophoresis). The PCR sample should be diluted 2- to 20-fold for optimal separation using E-Gel agarose gels.

Results

Platinum II *Taq* Hot-Start DNA Polymerase was used to amplify a 585 bp fragment from varying percentages of whole human blood (1–20%). Blood samples that were preserved with EDTA or citrate were added directly to the PCR reactions. During optimization, the $MgCl_2$ concentration was increased to 4.5 mM in the final reaction volume by adding 25 mM $MgCl_2$. The cycling protocol was: 1 cycle at 94°C for 2 min; 35 cycles at 94°C for 15 sec, 60°C for 15 sec, and 68°C for 15 sec. The products were separated using 1% agarose gels in TAE buffer. Results demonstrate that Platinum II *Taq* Hot-Start DNA Polymerase can successfully amplify a specific target from up to 20% whole blood that is stabilized with either EDTA or citrate. (Figure 1).

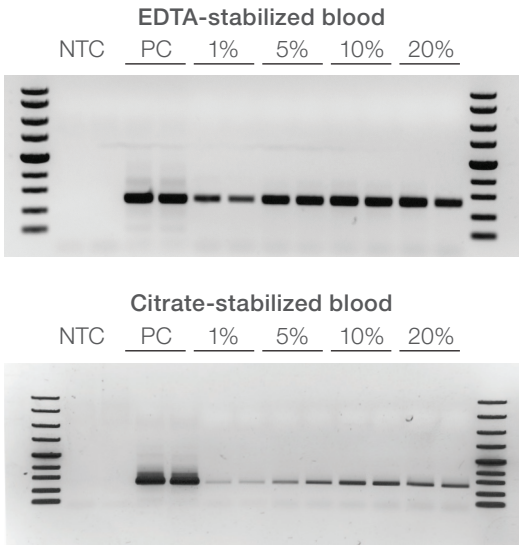

Figure 1. Direct amplification from whole blood. Amplification of a 585 bp fragment from varying percentages (v/v) of human blood was performed in duplicate using Platinum II *Taq* Hot-Start DNA Polymerase. $MgCl_2$ was added to 4.5 mM final concentration. Thermo Scientific™ ZipRuler™ Express DNA Ladder 2 was used as a size standard. NTC: no-template control, PC: positive control from 1 ng of purified human genomic DNA.

Summary

With its high tolerance to inhibitors and the unique composition of the Platinum II PCR buffer that allows annealing at 60°C for any primer pair, Platinum II *Taq* Hot-Start DNA Polymerase enables fast and convenient amplification of DNA directly from blood. This procedure can help streamline PCR workflows for multiple applications involving blood samples.

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

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