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It's the Joy of Discovery
Real Biotech Corporation
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AmpEasy™ Blood Direct PCR Kit

Description

AmpEasy™ Blood Direct PCR Kit is designed for performing PCR directly from whole blood without DNA purification or sample preparation. AmpEasy™ Blood Direct PCR Kit has been validated for the direct amplification of DNA fragments from whole blood, blood collected in different anti-coagulant tubes(such as EDTA, citrate or heparin), on FTA® Elute Cards, Whatman 903® Specimen Collection Paper ("Guthrie cards") or regular filter paper.

AmpEasy™ Blood Direct PCR Kit is optimized to give excellent results with blood originated from most of the mammalian animals. AmpEasy™ Blood Direct PCR Kit contains all components required for whole blood PCR except the template (whole blood) and primers. By using AmpEasy™ Blood Direct PCR Kit, DNA fragments may be amplified directly from reactions containing 1 - 25% (v/v) whole human blood without pretreatment of blood samples or DNA isolation, significantly reducing contamination risk, turnaround time and cost of genetic testing.

Features

- PCR can be performed directly from whole blood without DNA purification or sample preparation.
- Direct amplification of blood samples stored in different anti-coagulant tubes and from filter papers.
- No DNA purification is required, minimizing pipetting tasks and saving plenty of time.
- Immediate startup using optimized reagents and protocols.
- All components required for whole blood PCR are supplied except the template (blood) and primers.

Applications

- Ideal for analysis of large numbers of different blood samples
- DNA sequencing / Cloning / Genotyping
- Multiplex PCR / SNP detection / PCR-RFLP / Quantitative PCR
- Molecular diagnostic test
- Identity testing / Forensic DNA Analysis

Quality Control

Sensitivity and reproducibility of AmpEasy™ Blood Direct PCR Kits are tested in reproducibility assay: parallel 25μl reactions containing 1.5ul of human blood (concentration: 6%) and 1 μl of blood control primer mix. After 40 cycles, PCR products are verified by electrophoresis and DNA sequencing.

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Shipping and Storage Conditions

AmpEasy™ Blood Direct PCR Kit is shipped on dry ice and should be stored immediately upon receipt at -20℃ in a constant temperature freezer. With proper storage, AmpEasy™ Blood Direct PCR Kit can be stored for up to 12 months without showing any deduction in performance and quality.

Product Components and Storage Conditions

Cat. No.:	BDP100
Product Name:	AmpEasy™ Blood Direct PCR Kit
Size (25 µl/reaction):	100 preps
AmpEasy™ Blood DNA Polymerase	100 ul
2X AmpEasy™ Blood PCR Buffer*	1.25 ml
Blood Control Primer Mix (5 µM each)#	100 ul
$MgSO_4$ Solution	200 ul
EDTA Solution	200 ul

* Includes Mg^{2+} and dNTPs.

Contains blood control forward primer and blood control reverse primer (Size: 192 bp; Tm : 55℃)

Protocol

Sample Type: Whole Blood Collected in Anti-Coagulant Tubes

Important Notes Before Starting:

1. Suggested starting amount: 6% blood (can be added directly to the reaction without further modification). For example, add 1.5ul of 100% blood into 25ul reaction, the blood concentration will be 6%.
2. For blood concentrations greater than 20%, optimization of Mg^{2+} concentration may be required. $MgSO_4$ Solution and EDTA Solution are also provided for optimization of Mg^{2+} concentration. Please note that excess Mg^{2+} may result in spurious PCR products. Also if unspecific products are created, the effective Mg^{2+} concentration can be decreased by adding the chelating agent EDTA. Typically, adding 1-3ul of EDTA to a 25 µl reaction is sufficient to eliminate non-specific products.
3. Do not add Mg^{2+} and dNTPs to the PCR reaction since they are already included in 2X AmpEasy™ Blood PCR Buffer.
4. For GC-rich templates (CG percentage is greater than 70%), it's recommended to add 1-10% DMSO to the PCR reaction. Please note that if high DMSO concentration is used, the annealing temperature must be decreased, as DMSO alters the melting point of the primers. 10% DMSO decreases the annealing temperature by 5.5–6.0℃.

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Procedure:

1. Carefully mix and spin down all tubes before opening to ensure homogeneity and improve recovery. The PCR setup can be performed at room temperature. (This protocol serves only as a guideline for PCR amplification. Optional reaction conditions may vary and must be individual determined. Please note that the optimal conditions will depend on the primers, the percentage of blood in the reaction and/or the type anticoagulants used, since anticoagulants can alter the available $Mg2^+$ concentration.)

Component	25ul / prep	50ul / prep
2X AmpEasy™ Blood PCR Buffer*	12.5 µl	25 µl
Blood Control Primer Mix (5 µM each)	1 µl	2 µl
AmpEasy™ Blood DNA Polymerase	1 µl	2 µl
Whole Blood#	1.5 µl	3 µl
ddH_2O	Add to 25 µl	Add to 50 µl
Optional Components for Reaction Optimization		
$MgSO_4$ Solution§	1-3 µl	2-6 µl
EDTA Solution§	1-3 µl	2-6 µl
DMSO☆	1-10%	1-10%

* Includes Mg^{2+} and dNTPs.

Add 1.5ul of 100% blood into 25ul reaction, the blood concentration will be 6%. (best starting amount; no further modification is required)

§ For blood concentrations greater than 20%, optimization of Mg^{2+} concentration may be required.

☆ For GC-rich templates, it's recommended to add 1-10% DMSO to the PCR reaction.

2. Suggested reaction parameters are as below.

Segment	Number of Cycles	Temperature	Time	Cycle Step
1	1	95℃	10 minutes	Cell Lysis and DNA Extraction
2	40~45	95℃	30 seconds	Denaturation
		50~68℃#	30 seconds	Annealing
		72 ℃*	30 seconds	Extension
3	1	72 ℃	7 minutes	Final Extension
4	1	4 ℃	∞	Hold PCR product

Optimal annealing temperature is depending on user's primer sequences.

* For PCR products longer than 1 kb, use an extension time of approximately 1 min per kb DNA.

3. Place the PCR tubes or PCR plates in the thermal cycle and start the PCR program.

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Sample Type: Whole Blood Sample from FTA® Cards / Filter Papers

Due to the long-term stability of nucleic acids at room temperature, it's popular to preserve blood samples on filter papers. Filter papers usually contain a wide variety of chemical compounds that may inhibit PCR reaction, and special extraction protocol is often required before these blood samples can be used in PCR reaction. However, AmpEasy™ Blood Direct PCR Kit is especially designed to amplify DNA fragments directly from blood stored on FTA® cards / filter papers without prior DNA purification.

Important Notes Before Starting:

1. If blood dried onto a Whatman card is used, a 0.5 mm~1 mm in diameter punch can be added directly to the reaction without any pretreatment. The optimal reaction volume varies depending on the card type used. For Whatman 903 Card, optimal reaction volume is 10–50 µl; For Whatman FTA Elute Card, optimal reaction volume is 25–50 µl; For Whatman FTA Gene Card, optimal reaction volume is 50 µl.
2. To prevent cross-contamination between samples, it's very important to clean the cutting tool (ex: the Harris Uni-Core puncher) between each sample by dipping it into either 70 % EtOH or 2% bleach solution and press the plunger up and down at least 5 times. After cleaning, wipe the tip with a clean paper towel. The cutting mat must be rinsed with the same solution after each sampling.
3. For blood concentrations greater than 20%, optimization of Mg^{2+} concentration may be required. $MgSO_4$ Solution and EDTA Solution are also provided for optimization of Mg^{2+} concentration. Please note that excess Mg^{2+} may result in spurious PCR products. Also if unspecific products are created, the effective Mg^{2+} concentration can be decreased by adding the chelating agent EDTA. Typically, adding 1-3ul of EDTA to a 25 µl reaction is sufficient to eliminate non-specific products.
4. Do not add Mg^{2+} and dNTPs to the PCR reaction since they are already included in 2X AmpEasy™ Blood PCR Buffer.
5. For GC-rich templates (CG percentage is greater than 70%), it's recommended to add 1-10% DMSO to the PCR reaction. Please note that if high DMSO concentration is used, the annealing temperature must be decreased, as DMSO alters the melting point of the primers. 10% DMSO decreases the annealing temperature by 5.5–6.0℃.

Procedure:

1. Punch out 0.5-1 mm² of sample.【To prevent cross-contamination between samples, clean the cutting tool (ex: the Harris Uni-Core puncher) between each sample by dipping it into either 70 % EtOH or 2% bleach solution and press the plunger up and down at least 5 times. After cleaning, wipe the tip with a clean paper towel. The cutting mat must be rinsed with the same solution after each sampling.】
2. Put punch-out disc in a sterile PCR tube and rinse with 20ul of ddH_2O. Let stand for 3 minutes.

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3. Remove the ddH_2O by pipetting and add in following components. Carefully mix and spin down all tubes before opening to ensure homogeneity and improve recovery. The PCR setup can be performed at room temperature. (This protocol serves only as a guideline for PCR amplification. Optional reaction conditions may vary and must be individual determined. Please 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 $Mg2^+$ concentration.)

Component	25ul / prep	50ul / prep
2X AmpEasy™ Blood PCR Buffer*	12.5 µl	25 µl
Blood Control Primer Mix	1 µl	2 µl
AmpEasy™ Blood DNA Polymerase	1 µl	2 µl
Whole Blood Stored on FTA® Cards / Filter Papers #	0.5-1 mm²	0.5-1 mm²
ddH_2O	Add to 25 µl	Add to 50 µl
Optional Components for Reaction Optimization		
$MgSO_4$ Solution§	1-3 µl	2-6 µl
EDTA Solution§	1-3 µl	2-6 µl
DMSO☆	1-10%	1-10%

* Includes Mg^{2+} and dNTPs.

\# Recommended sample amount is 0.5-1 mm² punch.

§ For blood concentrations greater than 20%, optimization of Mg^{2+} concentration may be required.

☆For GC-rich templates, it's recommended to add 1-10% DMSO to the PCR reaction.

4. Suggested reaction parameters are as below.

Segment	Number of Cycles	Temperature	Time	Cycle Step
1	1	95℃	10 minutes	Cell Lysis and DNA Extraction
2	40~45	95℃	30 seconds	Denaturation
		50~68℃ #	30 seconds	Annealing
		72 ℃*	30 seconds	Extension
3	1	72 ℃	7 minutes	Final Extension
4	1	4 ℃	∞	Hold PCR product

\# Optimal annealing temperature is depending on user's primer sequences.

* For PCR products longer than 1 kb, use an extension time of approximately 1 min per kb DNA.

5. Place the PCR tubes or PCR plates in the thermal cycle and start the PCR program.

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Troubleshooting Guide

Whole Blood Collected in Anti-Coagulant Tubes	Whole Blood from FTA® Cards/Filter Papers
No PCR Product	
Make sure the blood sample is fresh or under proper storage conditions.	Punch out 0.5-1 mm² of sample.
Add 1.5ul of 100% blood into 25ul reaction, the blood concentration will be 6%. Starting blood concentration of 6% can be added directly to the PCR reaction without further modification.	Put punch-out disc in a sterile PCR tube and rinse with 20ul of ddH_2O. Let stand for 3 minutes. After removing the ddH_2O by pipetting, prepare the PCR reaction and run PCR directly.
Make sure that there are no pipetting errors.	
Use the control primers to perform the control reactions.	
Check primer design.	
Optimize annealing temperature according to the primer design.	
Make sure the anticoagulants chemicals are not excess.	
Increase the number of cycles. It's easier to perform PCR in a low blood concentration condition.	
For blood concentrations greater than 20%, optimization of Mg^{2+} concentration may be required. $MgSO_4$ Solution and EDTA Solution are also provided for optimization of Mg^{2+} concentration. For a 25ul reaction, adding 1ul $MgSO_4$ Solution is equivalent to 1mM $MgSO_4$ in final concentration. We recommend adding 1-3ul for modification. Please note that excess Mg^{2+} may result in spurious PCR products. Also if unspecific products are created, the effective Mg^{2+} concentration can be decreased by adding the chelating agent EDTA. Typically, adding 1-3ul of EDTA to a 25 μl reaction is sufficient to eliminate non-specific products.	
For GC-rich templates (CG percentage is greater than 70%), it's recommended to add 1-10% DMSO to the PCR reaction. Please note that if high DMSO concentration is used, the annealing temperature must be decreased, as DMSO alters the melting point of the primers. 10% DMSO decreases the annealing temperature by 5.5–6.0℃.	
Non-Specific Products with Discrete Bands	
Increase annealing temperature or perform a temperature gradient PCR to find the optimal temperature.	
Shorten extension time.	
Titrate the amount of blood in the reaction.	
Reduce the total number of cycles.	
Decrease primer concentration.	
Design new primers.	

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