Blood Direct PCR Kit

For direct PCR analysis of blood without DNA purification

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Blood Direct PCR Kit

Cat. No. 4992529/4992530

Kit Contents

Contents	4992529 20 µl ×100 rxn	4992530 20 µl ×500 rxn
2× Blood Direct PCR MasterMix	1 ml	5×1 ml
RNase-Free ddH_2O	1 ml	5×1 ml
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Storage

Blood Direct PCR Kit can be stored at -20°C for up to 12 months. 2× Blood Direct PCR MasterMix needs to be thawed and mixed by gentle inverting before use. For regular use, this kit can be stored at 2-8°C for up to 3 months. Please avoid repeated freezing and thawing.

Introduction

Blood Direct PCR Kit allows direct PCR analysis of blood sample, without the need of DNA purification and sample preparation. The PCR MasterMix supplied in this kit is a 2 × premix, with which only the addition of blood template and primers are required to start a PCR analysis. The PCR premix in this kit contains Taq DNA polymerase which is genetically modified towards specific stress resistance, and the PCR reaction system is also optimized according to this stress resistant enzyme. Blood samples which are stored by different methods can be used as templates to perform PCR using this kit. The 3' end of PCR product is dA, which allows the PCR product to be directly used in TA cloning.

This kit can efficiently amplify single copy gene in human genomic DNA, and could be widely used in research studies like genomic DNA fragment amplification (≤5 kb), high throughput genetic analysis and genotyping (like gene deletion).

Features

Simple and fast: DNA purification and sample preparation are not necessary, which makes the whole PCR process quick and convenient.

High inhibiter resistance: DNA polymerase supplied in this kit is genetically modified according to specific stress resistance, and the unique optimized buffer system can also strengthen its inhibiter resistance.

High compatibility: This kit can be used to amplify DNA fragment with high GC content and complicated secondary structure, and the length of DNA fragment can be up to 5 kb.

Wide applicability: This kit can be used to analyze blood samples from different species and under different storage conditions. (e.g. blood sample from human, mouse, rat or poultry; blood stored at 4°C or frozen blood; anti-coagulation blood with EDTA, citrate or heparin; liquefied blood clot and dry blood spots on whatman 903® or FTA® card.)

High throughput: This kit can be used with 96/384-well PCR plates for large-scale samples identification by PCR.

No contamination: Blood can be directly used as template without the need for pretreatment, so cross-contamination can be avoided.

Important Notes Before Starting

1. Excessive blood templates inhibit the PCR amplification. Please refer to "Recommended volume of blood template" to adjust the dosage of templates. In a 20 µl reaction system, the optimum template volume is 1-2 µl. In the initial experiment, it is recommended to set the gradient template dosage at the range of 1-5 µl to determine the optimum template dosage.
2. If using other reaction system, please decrease or increase the template volume proportionally.
3. Due to the denaturation of hemoglobin in the blood template, the amplified product might present turbidity, which does not affect the subsequent electrophoresis detection. However, further purification is needed for subsequent experiments such as cloning and sequencing.

Protocol

Note: The following protocol is just for case study, please setup the reaction condition according to specific circumstance (e.g. different structure of primers and template).

1. Use human anti-coagulation blood as template, perform a PCR reaction by using Blood Direct PCR Kit to amplify a 1,000 bp DNA fragment.
2. Setup the PCR reaction according to the following table.

Reaction system

Components	50 µl reaction	20 µl reaction	Final concentration
Blood template	2.5 µl	1 µl	—
Forward primer (10 µM)	1.25 µl	0.5 µl	250 nM
Reverse primer (10 µM)	1.25 µl	0.5 µl	250 nM
2× Blood Direct PCR MasterMix	25 µl	10 µl	1×
RNase-Free ddH_2O	to 50 µl	to 20 µl	—

Recommended volume of blood template (in 20 μl PCR reaction) :

Template type	Sample preparation method	Range of volume	Recommended volume
Human anti-coagulation blood	N/A	0.1-5 μl	1 μl
Mouse anti-coagulation blood	N/A	0.1-5 μl	1 μl
Poultry anti-coagulation blood	N/A	0.1-5 μl	1 μl
Human blood clot	Liquefaction (cat#RK165)	0.1-5 μl	1 μl
Dry blood spot	Punch by puncher	1-2 slides of Dry blood spot (3 mm diameter)	1 slide of Dry blood spot (3 mm diameter)
Cell culture	Centrifuge, remove the supernatant, then suspend by Buffer TE	1-10^3 cells	100 cells

3. Setup the PCR cycle according to the following table.

PCR cycle set-up:

Stage	Cycle	Temperature	Time	Step
Pre-denaturation	1×	95°C	3-5 min	Pre-denaturation
PCR reaction	35-40×	94°C	15 sec	Denaturation
		60°C	20 sec	Annealing
		72°C	1 min	Extension
Final extension	1×	72°C	5 min	Final extension

4. Result Detection: Take 10 μl reaction products for agarose electrophoresis detection.