Ver.EN20231030

Blood Advanced Direct PCR Kit

Product description

The Blood Advanced Direct PCR Kit is a kit that enables direct PCR amplification of whole blood samples without DNA purification or sample pretreatment. This kit is compatible with fresh blood containing conventional anticoagulants such as EDTA, heparin, citrate, frozen blood and commercial Whatman903™ and FTA™ dried blood stains. The kit contains a combination of genetically engineered DNA Polymerase with high fidelity and tolerance to PCR inhibitors. It can efficiently amplify 8 kb genomic fragments under rapid elongation conditions, for the fragments less than 2 kb, the extension time of 3-5 sec/kb can be set to complete the amplification, thus significantly reducing the identification and detection time of blood samples.

Hieff™ Advanced High-Fidelity DNA Polymerase Mix has a flat 3 'end of the amplified product, which is suitable for one-step rapid cloning and TOPO cloning (YEASEN Cat#10909/10910/10911/10922).

The Positive control primer mix (10 μmol/L each) provided in the kit is capable of amplifying a fragment 237 bp in length from the upstream conserved sequence of the *sox21* gene in mammals and most vertebrates, which could be used as a positive control.

Components

Components No.	Name	10188ES20	10188ES50	10188ES60	10188ES76
10188-A	2× Hieff™ Blood Advanced PCR Buffer	500 μL	1.25 mL	2.5 mL	12.5 mL
10188-B	Hieff™ Advanced High-Fidelity DNA Polymerase Mix	20 μL	50 μL	100 μL	500 μL
10188-C	Positive control primer mix (10 μM each)	50 μL	100 μL	200 μL	1 mL
10188-D	10× DNA loading buffer	200 μL	500 μL	1 mL	5× 1 mL

Specifications

Product specification	Kit
Hot Start	Built-in Hot Start
Overhang	Blunt
Conditions for transportation	Ice Packs
Product Type	Direct PCR Kit
Apply to (application)	Different types of whole blood samples

Storage

The products should be stored at -25~-15°C for one years. Please avoid repeated freeze-thaw.

Instructions

1. Reaction System

Components	Volume (μL)
ddH_2O	to 50 μL
2× Hieff™ Blood Advanced PCR Buffer	25 μL
Forward Primer (10 μmol/L)	2 μL
Reverse Primer (10 μmol/L)	2 μL
Hieff™ Advanced High-Fidelity DNA Polymerase Mix	1 μL
Blood sample	X μL

Table 1 Reaction system (50 μL)

[Note]:

1) All components should be thoroughly mixed before use.

2) Primer final concentration: The recommended final concentration for each primer is 0.4 μmol/L, too high will result in non-specific amplification.

3) Template usage: The optimal whole blood template concentration range is 0.5%-20%, and the recommended dosage is 10% as the initial trial condition, i.e. 5 μL whole blood in 50 μL reaction system as a template, taking care to avoid suction of blood clots. For dried blood stains stored on Whatman™ filter paper cards, a round paper of about 1 mm^2 with blood stains can be used for amplification without pretreatment and directly placed into the PCR reaction solution.

2. Reaction Program

Cycle steps	Temperature (°C)	Time	Cycles
Predenaturation	95	5 min	1
Denaturation	95	15 sec	30-35
Annealing	60	15 sec	
Extension	72	3-10 sec/kb	
Final extension	72	5 min	1

Table 2 Amplification protocol

[Note]:

1) Annealing temperature: please refer to the theoretical Tm value of the primer or 1-2°C below the primer Tm value. If amplification product specificity is poor, an annealing temperature gradient can be established to find optimal annealing conditions.

2) Extension time: Most of the target fragments below 8 kb could be amplified with the extension time of 10 sec/kb, and most of the fragments below 2 kb could be amplified with the extension time of 3-5 sec/kb. If the amplification efficiency is low, the time can be appropriately extended to 20-30 sec/kb and should not exceed 60 sec/kb.

3. Analysis of PCR Products

After the PCR reaction, it is recommended to centrifuge the reaction product at 4,000 rpm (1,000 x g) for 1-3 min to precipitate blood cell debris and remove the supernatant for downstream analysis. This step can eliminate the interference of multiple residual components in whole blood on electrophoresis detection, and is especially necessary for PCR products using high concentration of blood as template. Typically, 30 to 35 μL of supernatant is preferable for 5 μL/50 μL(10%) reaction product and 40 to 45 μL of supernatant is preferable for 3 μL/50 μL(5%)

reaction product. For other analyses of the PCR product, such as restriction enzyme digestion, the product should be diluted 2 to 4 times to reduce the interference of salts and other inhibitors in the reaction.

4. Control response

Positive Control Primer Mix (10 μmol/L each) was provided in the kit for positive control reactions. The 237 bp fragment upstream of the *sox21* gene can be amplified from the genome of mammals and many other vertebrates. The amplified region is a highly conserved non-region.

Primer F: 5'- AGCCCTTGGGGASTTGAATTGCTG -3'

Primer R: 5'- GCACTCCAGAGGACAGCRGTGTCAATA -3'

Notes

1. The recommended amount of blood template is 10% of the total reaction volume, i.e. 5 μL of whole blood in 50 μL reaction system as a template, taking care to avoid suction of blood clots.
2. Most of the target fragments below 8 kb could be amplified with the extension time of 10 sec/kb, and most of the fragments below 2 kb could be amplified with the extension time of 3-5 sec/kb. If the amplification efficiency is low, it can be used when extended to 30 sec/kb.
3. After PCR reaction, it is recommended to centrifuge the reaction product at 4,000 rpm (1,000 x g) for 1-3 min to precipitate blood cell debris and remove the supernatant for downstream analysis.
4. This product should not be used directly for medical diagnosis.
5. For your safety and health, please wear lab coats and disposable gloves for operation.
6. This product is for research use ONLY!