TOOLS BlooDirect PCR kit

For PCR amplification from whole blood without DNA isolation

Contents

Introduction

TOOLS BlooDirect PCR Kit allows amplification of DNA directly from whole blood containing EDTA without any DNA purification step. This kit is versatile and recommended for end-point PCR analysis of blood samples from various species and with different anticoagulants or storage conditions. With special formulated Blood PCR reaction buffer, this kit exhibits high resistance to PCR inhibitors found in blood and retains polymerase activity even with samples containing 30% whole blood. The setup of a genotyping experiment with TOOLS BlooDirect PCR Kit cannot be simpler anymore: add the respective blood sample into reaction mix and run PCR right away.

The novel Blood Nova DNA polymerase in this kit is one of the thermostable DNA polymerases with strong 3'-5' exonuclease activity (proofreading activity), which results in its extreme high fidelity, 10-15 times higher than Taq DNA polymerase. So, PCR product from TOOLS BlooDirect PCR Kit is also great for cloning or other demanding applications.

Important Notes

1. The recommended starting amount is 5% blood added directly to the reaction without further modification.
2. The annealing rules are different from many common DNA polymerases (such as Taq DNA polymerases). Read protocol carefully before you begin the setup.
3. For extension, use 15 s for amplicons ≤1 kb or 30 s/kb for amplicons >1 kb.
4. After PCR, spin the reactions at 1000 × g for 1−3 minutes to pellet debris from blood.
5. Blood Nova DNA polymerase produce blunt end PCR products.

Materials needed but not supplied with the product

- 10 mM dNTPs
- DNase-free water

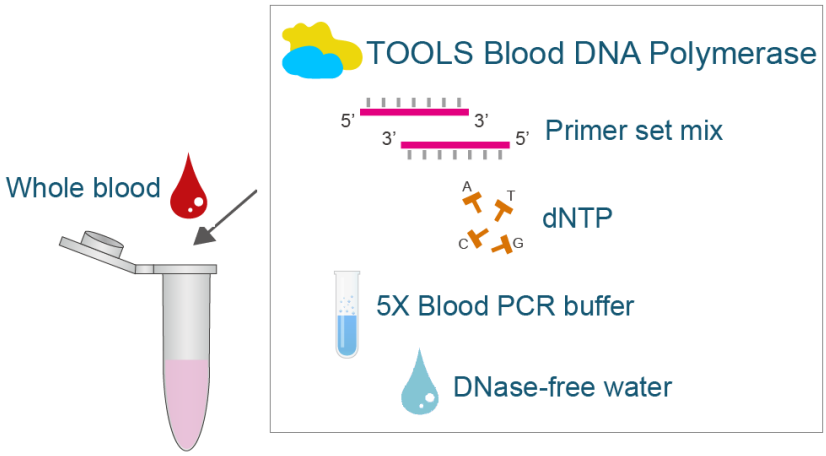
Kit Contents

Contents	TTG-BP01 (200 rxn)
Blood DNA polymerase (2000 U/mL)	200 µL
5X Blood PCR buffer	2 mL
Human control primer mix (10 µM each)	200 µL

Storage

All components of the kit should be stored at -20°C.

Work Flow



Prepare the PCR reaction

Perform PCR

Downstream assays

Protocol

1. Prepare the PCR reactions by the below table

Component	50 µL rxn	Final Conc.
TOOLS Blood DNA Polymerase	1 µL	2 U
5X Blood PCR buffer	10 µL	1X
Forward & Reverse primer mix (2 µM each)	5 µL	0.2 µM each
10 mM dNTPs	1 µL	0.2 µM
Whole blood sample	X µL	5 – 40 %
DNase-Free ddH_2O	X µL	-

Note:

a. Adjust the volume of blood and DNase-free water according to the blood concentration in reaction by the following table

Blood conc. in rxn	Blood sample (µL)	DNase-free water (µL)	Total
5%	2.5	30.5	33 µL
10%	5	28	
20%	10	23	
30%	15	18	
40%	20	13	

b. The recommended starting point is 5%. In general, if higher blood percentage (>20%) is used, higher reaction volume (up to 50 µL) is recommended. For small amplicons, whole blood up to 40% can be used.

2. Mix the reaction thoroughly by brief vortex.

3. (Optional) Human control primer mix is designed primers in H2O that amplify a 324 bp fragment of human genomic DNA. The amplified region is Human Dystrophin gene. Each primer concentration in mix is 10 µM. Prepare the control PCR reaction by the following table:

Component	50 µL rxn	Final Conc.
TOOLS Blood DNA Polymerase	1 µL	2 U
5X Blood PCR buffer	10 µL	1X
Human control primer mix	1 µL	0.2 µM each
10 mM dNTPs	1 µL	0.2 µM
Whole blood sample	X µL	Following step 1.
DNase-Free ddH_2O	X µL	

4. Perform PCR using the recommended programs outlined below. Choose the program according to the length of amplicon:

For amplicon ≤1 kb

Stage	Cycle	Temp. (℃)	Time	Step
Initial denaturation	1	98	2 mins	Initial denaturation
PCR	35	98	10 sec	Initial denaturation
		60	30 sec	Annealing
		72	15 sec	Extension
	1	72	2 min	Complete extension
Hold	1	4	-	-

For amplicon >1 kb

Stage	Cycle	Temp. (℃)	Time	Step
Initial denaturation	1	98	2 mins	Initial denaturation
PCR	35	98	10 sec	Initial denaturation
		60	30 sec	Annealing
		72	30 sec	Extension
	1	72	2 min	Complete extension
Hold	1	4	-	-

Note: The initial denaturation step at 98 °C also allows the lysis of leukocytes, making genomic DNA available for PCR. This is essential step, so please do not alter the condition.

5. After amplification, centrifuge the PCR reaction at 1000 × g for 1−3 minutes to collect the supernatant for subsequent analysis, e.g., gel electrophoresis.

Note: This step separates the various components of blood. This is especially important when high blood concentrations are used, as there can be a substantial amount of cell debris, etc. in the tube after the PCR reaction.

If post-PCR enzyme treatment is required (e.g., PCR-RFLP or cloning construct), it may be necessary to dilute the PCR product 2−4-fold to dilute the salts and other inhibitors.

The product is for research only; it is not for diagnostic or clinical use.