5. Result detection: After the reaction, 5μL reaction product was taken and electrophoresis buffer was added to detect the result.

This product is for scientific research only, which shall not be used for clinical diagnosis or other purposes.

mTaq DNA Polymerase

Cat. No. : CW0749M

Shipping and Storage : -20°C

Components

Component	CW0749M 2500 U
mTaq DNA Polymerase（5 U/μL	5×100 μL
mTaq PCR Buffer, 10×	5×1.8 mL

Note: mTaq PCR Buffercontains 30 mM $MgCl_2$

Principle

mTaq DNA Polymerase is a new type of DNA Polymerase which is modified by deletion of an amino acid segment in the N terminal of Taq DNA polymerase and mutation. Modified to tolerate inhibitors present in whole blood, the product can directly amplify DNA in whole blood samples of humans and mice without prior genome extraction and purification. This product can be used directly for T/A cloning because the 3' end of the amplified PCR product has an "A" base.

Activity Definition

After column purification, the purity was more than 99% according to SDS-PAGE. No exogenous nuclease activity was detected. No host residual DNA was detected by PCR. Can effectively amplify single copy genes in human genome; Stored at room temperature for one week, no obvious change in activity.

Procedure

1. Reverse the mTaq DNA Polymerase repeatedly until it is thoroughly mixed before use.
2. Put the PCR thin-walled tube on ice and add the following reagents except the whole blood. 1.PCR Reaction System

Reagent	50 uL Reaction System	Final Conc.
mTaq DNA Polymerase	1 uL	
mTaq PCR Buffer, 10×	5 uL	1×
dNTP Mix, 2.5 mM each	4 uL	200 uM each
Forward Primer, 10 uM	2 uL	0.4 uM
Reverse Primer, 10 uM	2 uL	0.4 uM
*Whole Blood	≤10%	
RNase-Free water	× uL	
Total	50 uL	

Note:1) * Before adding the whole blood, suck up and down repeatedly and mix all kinds of reagents thoroughly.

2) DNA template: Whole blood can be treated with heparin sodium, Na-EDTA, K-EDTA, or sodium citrate. A whole blood level of 5-10% is usually recommended. High concentrations of blood are not recommended. For templates with high GC content, 10%DMSO was added.

3) Primers: The length of oligonucleotide primers usually contains 20-30 nucleotides, and the optimal GC content is 40-60% and evenly distributed in the primers. In conventional PCR reactions, primer concentrations should be 0.1-1.0 μM as a reference for the set range.

3. Finally, add the whole blood to the bottom of the tube.
4. PCR reaction conditions

Step	Temperature	Time	
Initial denaturation	95 °C	5 mins	
Denaturation	95 °C	30 s	35-40 cycles
Annealing	50-68 °C	30 s	
Extension	72 °C	250-500 bp/min	
Final Extension	72 °C	10 mins	

Note: 1) Preheat the PCR instrument to 94-95°C, place the sample on the PCR instrument and start the cycle.

2) mTaq improves cold sensitivity and has some hot start characteristics. Reaction ingredients can usually be prepared on ice, and finally

Nonspecific products were avoided by adding polymerase and preheating the thermocycler to denaturation temperature (95°C).

3) Denaturation temperature and time: In order to fully lysate blood cells and release/denaturate DNA prior to PCR cycle, the initial denaturation is required to be 95°C for 5 minutes.

4) Annealing temperature and time: the annealing time is usually 30 seconds -1 minute. The annealing temperature can be 5°C lower than the theoretical annealing temperature (Tm) and optimized by gradient PCR.

5) Elongation time: the elongation reaction is usually carried out at 72°C. Generally, the stretch time is 1 minute every 250-500 bp. The final extension is recommended at 72°C for 10 minutes.

6) Usually 35-40 cycles can achieve optimal amplification.