mTaq DNA Polymerase

Item No. M665697 (100 rxns)
Storage condition: -20℃

Product content

individual parts making up a compound	100 rxns
mTaq DNA Polymerase, 5 U/μl	5×100 μl
mTaq PCR Buffer, 10×	5 x 1.8 ml

Product Introduction

mTaq DNA Polymerase is a new type of DNA polymerase that has been modified by deleting an amino acid from the N-terminal end of Taq DNA Polymerase and mutating it. This modification makes the product tolerant to inhibitors present in whole blood and enables direct amplification of DNA from human and mouse whole blood samples without the need for prior genome extraction and purification. The 3' end of the PCR product is A, which can be used directly for T/A cloning.

Quality control

After several column purifications, the purity of the product is greater than 99% by SDS-PAGE; no exogenous nuclease activity is detected; no host residual DNA is detected by PCR; it can effectively amplify single-copy genes in the human genome; and there is no obvious change in the activity after storing it at room temperature for one week.

Usage

1. Before use, invert the mTaq DNA Polymerase repeatedly until it is completely mixed.
2. Place the PCR thin-walled tube on ice and add the following reagents except whole blood.

reagents	50 μl reaction system	final concentration
mTaq DNA Polymerase	1 μl	/
mTaq PCR Buffer, 10×	5 μl	1×
dNTP Mix, 2.5 mM each	4 μl	200 μM each
Forward Primer (10 μM)	2 μl	0.4 μM
Reverse Primer (10 μM)	2 μl	0.4 μM
Whole blood*	≤10%	/
RNase-Free water	x μl	/
Total	50 μl	/

Attention:
1)*Repeatedly suck and beat up and down to completely mix the various reagents before adding whole blood,
2) DNA template: Whole blood can be treated with sodium heparin, Na-EDTA, K-EDTA or sodium citrate. A level of 5-10% whole blood is usually recommended. High blood concentrations are not recommended. For templates with high GC content, add 10% DMSO.
3) Primers: Oligonucleotide primers usually contain 20-30 nucleotides in length, and it is preferable that the GC content is 40-60% and evenly distributed in the primers. In the routine PCR reaction, please use the final concentration of 0.1-1.0 μM as a reference for setting the range of primer concentration.
3. Finally, whole blood is added to the bottom of the tube.
4. PCR reaction conditions

move	temp	timing	/
premutability	95° C	5 min	/
denaturation	95° C	30 s	35-40 cycles
annealing (metallurgy)	50-68° C	30 s	35-40 cycles

reach	72° C	250-500 bp/min	35-40 cycles
ultimate extension	72° C	10 min	/

Attention:

1) Preheat the PCR instrument at 94-95° C and place the samples on the PCR instrument to start the cycle.

2) mTaq has improved cold sensitivity and has some hot-start properties. Often, non-specific products can be avoided by preparing the reaction components on ice, adding the polymerase last, and preheating the thermal cycler to denaturing temperature (95° C) immediately after the reaction.

(3) Denaturation temperature and time: In order to fully lysed blood cells and release/denature DNA before PCR cycling, the initial denaturation is required to be 95° C for 5 minutes.

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

5) Extension time: extension reactions are usually performed at 72° C. The general extension time is 1 minute per 250-500 bp. The final extension is recommended to be carried out at 72℃ for 10 minutes.

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

5. Detection of results: At the end of the reaction, 5 μl of the reaction product was taken and added to the electrophoresis buffer to detect the results.