GCC BIOTECH®

DeltaQ Polymerase

 # G7126 250 units

Store at -20°C

Spin tubes briefly before use

Catalogue Number	Pack Size	☑
G7126	250 Units	✓
G7126A	500 Units	

Introduction

DeltaQ polymerase is a truncated version of Taq DNA Polymerase, lacking the first 278 amino acids. DeltaQ polymerase also contains mutations that make it resistant to inhibitors present in whole blood. DeltaQ polymerase tolerates up to 10% whole blood. For enhanced detection capacity, a single strand binding domain has been fused with the polymerase. DeltaQ polymerase is able to amplify up to 2 kb of lambda DNA. DeltaQ polymerase is mainly important for amplification from very low amount of template.

Guidelines for using DeltaQ Polymerase

DeltaQ Polymerase is provided with 10x DeltaQ reaction buffer. DeltaQ polymerase is supplied at conc of 1U/ λ. 1U is recommended for 50 ul final PCR reaction master mix.

Package information

Component	G7126	G7126A
DeltaQ (1 unit/λ)	250 Unit	(2X 500 Unit)
10X DeltaQ Reaction Buffer	1.25ml	(4X 1.25 ML)
30mM MgCl2	(1X 1.25 ml)	(4X 1.25 ML)
Control DNA template	10 µl	10 µl

Unit Defination

One unit incorporates 10nmol of deoxy-ribonucleotide into acid-insoluble product in 30 minutes at 74°C. Unit assay conditions: 25 mM TAPS (pH 9.3), 50 mM KCl, 2 mM MgCl2, 1 mM DTT, 0.2 mM dATP, dCTP, dGTP, dTTP utilizing M13mp18DNA as template.

Enzyme

The optimal amount of enzyme is 1µl in a 50 µl reaction. For PCR from tough sample or crude sample enzyme requirement need to be optimized. When cloning fragments amplified with DeltaQ Polymerase an extra adenine (A) is incorporated at the 3' end of the amplified PCR product. The 'A' overhang is required to perform TA cloning.

Table 1: PCR Mix preparation

Component	50 µl Reaction	Final conc.
Template DNA	X µl	<10 ng
10x Reaction Buffer	5µl	1X
30mM $MgCl_2$	5µl	3mM
10 mM dNTPs*	1 µl	200µM each
Forward primer#	1 µl	0.2 µM
Reverse primer#	1 µl	0.2 µM
H_2O	upto 50 µl	
DeltaQ Polymerase	1 µl	1U

Table 2: Cycling Instructions

Cycle step	2-step Protocol		Cycles
	Temp.	Time	
Initial Denaturation	94^0C	5min	1
Denaturation	94^0C	15-30sec	25-45
Annealing	Tm	15-60sec	
Extension	$68\text{-}72^0C$	30-60sec/1kb	
Final extension	$68\text{-}72^0C$	5-10min	1

Template

For low complexity DNA (e.g. plasmid, lambda or BAC DNA):0.1 pg–10 ng per 25 µl reaction volume PCR from low amount template requires higher no of cycles. For high complexity genomic DNA, the amount of template DNA should be 1–10 ng per 25 µl reaction volume. If cDNA synthesis reaction mixture is used directly as a source for the template, the volume used should not exceed 10 % of the final PCR reaction volume.

Primers

The recommended final primer concentration is 0.5 µM. If required, the primer concentration may be optimized between 0.2−1.0 µM. The results from primer Tm calculations can vary significantly depending on the method used. Always use the Tm calculator and instructions from reputed website to determine the Tm values of primers and optimal annealing temperature. If two-step PCR protocol is used, where both primer annealing and extension occur in a single step at 72°C, the primers should be designed accordingly.

• Use 94°C for denaturation.	**• Use 200 µM of each dNTP. Do not use dUTP.**
• The annealing temp depends on the primerTm	• Use 30-60ss/kb for extension.
• Use 0.5 µl of enzyme per 25 µl reaction and 1 µl per 50 µl reaction.	**Note:** DeltaQ DNA Polymerase produces A overhang at the 3' end.

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*Mg2+ and dNTP

DeltaQ reaction buffer is optimized to work well for most amplicons The 30 mM $MgCl_2$ conc. is provided separetly. For PCR from EDTA containing Blood sample, final amount of MgCl2 in reaction mixture need to be optimized. High quality dNTPs should be used for optimal performance with DeltaQ Polymerase. The polymerase cannot incorporate dUTP-derivatives or dNTP in the template strand so use of these analogues or primers containing them is not recommended. For optimal results always use 200 µM of each dNTP.

DMSO

The recommended reaction conditions for GC-rich templates include 3-5% DMSO as a PCR additive, which aids in the denaturing of templates with high GC contents. For further optimization the amount of DMSO should be increased in 2% increments. In some cases DMSO may also be required for supercoiled plasmids to relax for denaturation. Other PCR additives such as formamide, glycerol, and betaine are also compatible with DeltaQ Polymerase. If high DMSO concentration is used, the annealing temperature must be decreased, as DMSO affects the melting point of the primers. It has been reported that 10% DMSO decreases annealing temperature by 5.5–6.0°C.

Troubleshooting

Optimize annealing temperature.	**Increase extension time.**
• **Repeat and make sure that there are no pipetting errors.**	• Increase the number of cycles.
• **Make sure the cycling protocol was performed as recommended.**	• Titrate DMSO (2−8 %) in the reaction.
• **Use fresh high-quality dNTPs.**	• Denaturation temperature may be too low. Optimal denaturation temperature for most templates is 94-98°C.
• **Do not use dNTP mix or primers that contain dUTP or dITP.**	• Optimize denaturation time.
• **Titrate template amount. Template DNA may be damaged. Use freshly prepared template.**	• Check the purity and concentration of the primers. • Check primer design.
Non-specific products - High molecular weight smears	
• **Make sure the extension time used was not too long.**	• Titrate template amount.
• **Reduce the total number of cycles.**	• Optimize denaturation temperature.
• **Increase annealing temperature or try 2-step PCR protocol.**	• Decrease primer concentration.
Non-specific products - Low molecular weight discrete bands	
• **Increase annealing temperature**	• Titrate template amount.
• **Make sure the extension time used was not too long.**	• Decrease primer concentration. • Design new primers.

Component	Volume
Control DNA template	0.5µl
Control primer Mix	0.5 µl
10mM dNTPs	0.5 µl
Prime Taq DNA Polymerase	0.5 µl
Prime Taq Reaction Buffer	2.5 µl
$MgCl_2$	2.5 µl
Nuclease-free water	Upto25 µl

Control PCR set-up

Cycling conditions	
Step 1	94˚C – 5 min
Step 2	94˚C – 30 s
Step 3	57˚C – 30 s
Step 4	72˚C – 4 min
Step 5	72˚C – 10 min
Step 6	4 ˚C – Hold

Repeat Steps 2 to 4 for 25 Cycles. A ~2kb band will be obtained in Control PCR reaction.