Hot Start G9 Taq DNA Polymerase

 # G7120A 100 units

Store at -20°C

Spin tubes briefly before use

Catalogue Number	Pack Size	☑
G7120	100 Units	
G7120A	500 Units	✓

Introduction

Hot Start (HS) G9 Taq DNA Polymerase is an aptamer based hot start Taq DNA polymerase useful for preventing or reducing non specific DNA amplification in PCR. The aptamer inhibits the activity of Taq DNA polymerase at room temperature (below 45^{0}C), thus avoiding any extension due to non-specific annealing of primers at room temperature. This HS G9 Taq DNA polymerase is superior to any antibody-based or chemically modified hot-start DNA polymerase due to its longer stability and complete suppression of the enzyme activity at room temperature following the activation step.

Package information

Component	G7120A
Hot Start G9 Taq DNA Polymerase (2.5 unit/ul)	100 units X 5
10X HS Taq Buffer	225 ul X 5
25 mM $MgCl_2$	225 ul X 5
Control DNA tempalate	10ul
Control Primer mix	10 ul

Guidelines for using Hot Start G9 Taq DNA Polymerase

HS G9 Taq DNA Polymerase is provided with 10x HS G9 Taq DNA Reaction Buffer. The buffer contains no $MgCl_2$. A separate tube of $MgCl_2$ (25 mM) is provided to maintain the flexibility

Unit definition

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 0.5 µl in 25 µl reactions.
*HS G9 taq DNA Polymerase shows 5´→ 3´ polymerizing activity and 3'→5' exonuclease activity.

Table 1. Pipetting instructions (add items in this order).

Component	25 µl Reaction	Final conc.
Template DNA	X µl	<500 ng
10x Reaction Buffer	2.5µl	1X
10 mM dNTPs	0.5 µl	200µM each
Forward primer#	0.5 µl	0.2 µM
Reverse primer#	0.5 µl	0.2 µM
$MgCl_2$#	2.5 µl	2.5 mM
H_2O	To 25 µl	
HS G9 Taq	0.5 µl	1.25 U

In some template to get optimum result primers conc and MgCl2 conc. needed to be optimized.

Protocol

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Place a thin-walled PCR tube on ice and add the components as mentioned in Table 1 for each 25 µL reaction.
3. Mix the samples and spin down as quickly as possible.
4. Perform PCR using recommended thermal cycling conditions (see Table 2). For higher amplification (>4 Kb) extension at 68^{0}C is recommended.

Table 2. Cycling Instructions

Cycle step	2-step Protocol		Cycles
	Temp.	Time	
Initial Denaturation	94^{0}C	5min	1
Denaturation	94^{0}C	15-30sec	25-35
Annealing	Tm	15-60sec	
Extension	68^{0}C-72^{0}C	60sec/1kb	
Final extension	68^{0}C	5-15 min	1

Note

• Use 95°C for denaturation.	**• Use 200 µM of each dNTP. Do not use dUTP.**
• The annealing temp depends on the PrimerTm	• Use 30–60s/kb for extension.
• Gently vortex and briefly centrifuge all solutions after thawing.	

Template

For low complexity DNA (e.g. plasmid, lambda or BAC DNA): 1 pg–10 ng per 25 µl reaction volume or 2 pg− 20 ng per 50 µl reaction volume. For high complexity genomic DNA, the amount of DNA template should be 10–400 ng per 25 µl reaction volume or 20−800 ng per 50 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 recommendation for final primer concentration is 0.2 µM. If required, the primer concentration may be optimized between 0.1−1.0 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 G9rs and optimal annealing temperature. If using a two-step PCR protocol, where both primer annealing and extension occur in a single step at 72°C, the primers should be designed accordingly.

Mg2+ and dNTP

well for most amplicons. Standard concentration of MgCl2 in PCR reaction mixture is 2.5mM but the optimum concentration might vary between 1-5 mM. To keep this flexibility 25 mM MgCl2 is provided separately with 10X reaction buffer. High quality dNTPs should be used for optimal performance with HS G9 Taq DNA Polymerase. The polymerase cannot read dUTP-derivatives or dITP in the template strand so the 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. 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 HS G9 Taq DNA 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.**
• **Optimize annealing temperature.** • **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 dNTP.**	• 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
2X G9 Taq PCR Master Mix	25 µl
Control DNA template	1 µl
Control primer Mix	1 µl
Nuclease-free water	23 µl

Control PCR cycling conditions

Cycling conditions	
Step 1	94˚C – 5 min
Step 2	94˚C – 30s
Step 3	57˚C – 30s
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 band ~2kb will be obtained in control PCR.