GPV8 HF DNA polymerase

# PM06100	100 units	40ul
# PM06200	200 units	80ul
# PM06500	500 units	200ul

STORAGE AND PACKAGE INFORMATION

Shipping on gel ice. Upon arrival, store at -20℃, stable for one year

GPV8 HF DNA polymerase

10×PCR Buffer (200mM Tris-HCl (pH 9.0)，200mM KCl，100mM(NH4)2SO4，20mM MgCl2)

INTRODUCTION

For high speed and high performance PCR, the GPV8 HF DNA polymerase offers extreme performance. The GPV8 HF DNA polymerase brings together a novel Pyrococcus-like enzyme with a processivity-enhancing domain. It can generates long templates with an accuracy and speed, even on the most diffcult templates.

The error rate of GPV8 is determined to be 50-fold lower than that of Taq DNA polymerase, and 6-fold lower than that of pfu DNA polymerase.

The GPV8 HF DNA polymerase possesses the following activities:5′→3′ DNA polymerase activity and 3′→5′ exonuclease activity. It generates blunt ends in the amplification products.

- Use GPV8 DNA Polymerase at 0.5-2.5 U per 50 µl reaction volume. Do not exceed
- Use 15-30 s/kb for extension. Do not exceed 1 min/kb
- Use 98°C for denaturation
- Use 200 µM of each dNTP. Do not use dUTP
- GPV8 DNA Polymerase produces blunt end DNA products

DETECTION OF QUALITY CONTROL

Unit definition: One unit is defined as the amount of enzyme that will incorporate 10 nmoles of dNTPs into acid-insoluble form at 74°C in 30 minutes under the stated assay conditions.

DNA amplification assay: Performance in PCR is tested in amplification of 7.5 kb genomic DNA.

Exonuclease activity: Incubation of 10 U for 4 hours at 72°C in 50 µl assay buffer with 1 µg supercoiled plasmid is tested, released < 1%.

Genomic contamination assay: 30 cycles of PCR with 16s primers of E.coli is tested, no bands is amplified.

PROTOCOLS

TABLE 1. PIPETTING INSTRUCTIONS (IN ORDER)

Conponents	Volumes	Final concentration
GPV8 DNA Polymerase	1 ul	2.5U
10×HF Buffer	5 ul	1×
Primer（10 uM）	2.5 ul	0.5 uM
dNTP （10 mM）	1 ul	0.2 mM
Template	Variable	As required
ddH2O	Up to 50 ul	-

TABLE 2. CYCLING INSTRUCTIONS

Temp.		Time
98 ℃		30-180 s
98 ℃	20~30 Cycles	5-10 s
45~72 ℃		10-30 s
72 ℃		15-30 s/kb
72 ℃		5~10 min
4-12 ℃		∞

Temp.	Time
98 ℃	30-180 s
98 ℃	5-10 s
72 ℃	10-30 s per kb
72 ℃	5~10 min
4 ℃	∞

GENERAL GUIDELINES

Template : use of high quality, purified DNA templates greatly enhances the success of PCR. 10ng plasmid or viral, 50-200ng genomic were recommended for a 50 µl reaction.

Primers : oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%. while 0.5 µM is recommended.

Denaturation : an initial denaturation of 30 seconds at 98°C is sufficient from pure DNA templates. Longer denaturation times can be used (up to 3 minutes) for templates that require it. During thermocycling, the denaturation step should be kept to a minimum. Typically, a 5–10 second denaturation at 98°C is recommended for most templates.

Annealing : annealing temperatures required for use with GPV8 tend to be higher, Tm+3°C is recommended. For high Tm primer pairs, two-step cycling without a separate annealing step can be used.

Extension : the recommended extension temperature is 72°C. Extension times are dependent on amplicon length and complexity. Generally, an extension time of 15 seconds per kb can be used. For complex amplicons, such as genomic DNA, an extension time of 30 seconds per kb is recommended. Extension time can be increased to 40 seconds per kb for cDNA templates, if necessary.

NOTES

- **Research use only** these products are intended for research purposes by qualifed persons.
- Repeated freezing and thawing of the buffer can result in the precipitation or accumulation of $MgCl_2$ in insoluble form. For consistent results heat the buffer and vortex prior to use.