GDP-HiFi DNA Polymerase

Cat No. MB601-0100
Size: 100 Reactions
Conc. 1 U/µl
Store at -20°C

Description

GDP-HiFi is a new recombinant enzyme with genetic modification for its amino acid sequence, which results 70 times better fidelity than *Taq* DNA polymerase and an extremely fast elongation rate (as fast as 15 seconds per kb). GDP-HiFi has higher stability at high temperature. Users may program the initial denaturizing at even 100°C for 2 min, and this property makes GDP-HiFi perform very well for GC-rich PCR.

This special enzyme has been modified genetically and needs less concentration of magnesium than other polymerases. The suggestion for magnesium ion in the reaction is 0.8 to 1.2 mM. 10X GDP-HiFi PCR buffer contains no magnesium. A tube of 25-mM $MgSO_4$ is provided. Further optimization can be achieved for different targets of DNAs.

Reagents are provided for 100 PCRs of 50 µl each.

Note

- 10X GDP-HiFi PCR buffer contains BSA; store at –20°C.
- GDP-HiFi DNA Polymerase produces blunt-end PCR products. Add conventional *Taq* polymerase at the final step for further TA or GC cloning application.

Component 100 Rxn Kit

GDP-HiFi DNA Polymerase (1 U/µl)	100 µl
10X GDP-HiFi PCR buffer A (pH 8.1)	1 ml
10X GDP-HiFi PCR buffer B (pH 8.7)	1 ml
25 mM Magnesium Sulfate	1 ml
dNTP Mix (2mM each)	1 ml
DMSO	1 ml

GDP-HiFi DNA Polymerase Storage Buffer

50 mM Tris-HCl (pH 8.1), 60 mM KCl, 0.1 mM EDTA, 1 mM DTT, stabilizers, and 50% (v/v) glycerol.

Unit Definition

One unit of GDP-HiFi DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-insoluble material in 30 min at 74°C.

General Recommendations and Guidelines for PCR

General PCR parameters and troubleshooting information are documented in Innis, et al (Innis et al., 1990).

Template

GDP-HiFi DNA Polymerase is suitable for amplifying targets up to 10 kb from the following templates:

	Template Amount
Genomic DNA:	10–200 ng
Plasmid DNA :	1–5 ng
DNA :	~100 ng starting total RNA

Amplification of longer targets (up to 10 kb) may be possible, but may require more template and longer elongation times.

Primers

Use 0.3 µM per primer as a general starting point. For larger amounts of template (e.g., 200 ng genomic DNA), increasing the concentration up to 0.5 µM per primer may improve yield.

Annealing Temperature

The annealing temperature is slightly higher than with typical PCR. The optimal annealing temperature should be ~2°C lower than the Tm of the primers used. A range of 58–68°C is recommended. $MgSO_4$: $MgSO_4$ is not included in the 10X GDP-HiFi PCR buffer. Suggested working concentration is 1 mM, which is sufficient for most templates. For further optimization, modify the amount of $MgSO_4$ for final 0.8-1.2 mM of the concentration. Extension Time: As little as 15 seconds per kb may be used; 30 seconds per kb is suitable for most targets. Use up to 60 seconds per kb for maximum yield.

PCR Protocol

The following procedure is suggested as a starting point when using GDP-HiFi DNA Polymerase in any PCR amplification.

1. Program the thermal cycler as follows:
   Initial denaturation: 96~98°C for 2 minutes
   35 cycles of:
      Denaturation: 94°C for 15 seconds
      Annealing: 60–68°C (Tm of primers minus 2°C) for 10–30 seconds
      Extension: 68°C for 30–60 seconds per kb of PCR product
      Final extension: 68°C for 5 minutes
2. Add the following components to an autoclaved microcentrifuge tube at room temperature (for multiple reactions, prepare a Master Mix of common components to enable accurate pipetting):

Component	Volume	Final Conc
10X GDP-HiFi PCR Buffer (pH 8.1 or 8.7)	5 µl	1X
2 mM dNTP Mix	5 µl	0.2 mM each
2 mM $MgSO_4$	2 µl	1 mM
Primer mix (10 µM each)	1.5 µl	0.3 µM each
Template DNA (see page 2 for amounts)	≥1 µl	As required
GDP-HiFi DNA Polymerase (1 U/µl)	1 µl	1 U
DMSO (for GC-rich target)		5~10%
Autoclaved, distilled water		to 50 µl

3. Cap the tube, tap gently to mix, and centrifuge briefly to collect the contents.
4. Place the tube in the thermal cycler and run the program from
   Step 1. After cycling, maintain the reaction at 4°C. Samples can be stored at –20°C until use.
5. Analyze products using standard agarose gel electrophoresis.

Quality Control

GDP-HiFi DNA Polymerase is tested in a PCR functional assay, a doublestrand-endonuclease assay, and a 5'-exonuclease assay.

Other GDP-HiFi Product

GDP-HiFi Hot-Start
GDP-HiFi XL Hot-Start (for large size PCR amplification)

Other GDP-HiFi PCR Cloning Product

pClone-1	Blunt PCR cloning vector kit (direct blunt cloning)
pClone-1	GC PCR cloning vector kit (GC cloning)
pClone-1	C/T PCR cloning vector kit (GC/AT clonings at one shot)
pClone-Toxic	(PCR cloning for toxic gene to E. coli host)
pClone-XL	(PCR cloning for lager fragment)
pClone-T7	(PCR cloning for direct T7 expression)