



GDP-HiFi DNA Polymerase

Cat. No.: MB601-0100

Size: 100 reactions

Cat. No.: MB601-0010

Size: 10 reactions

Concentration of GDP-HiFi DNA polymerase: 1 U/ μ l

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 kilo base, kb). GDP-HiFi has higher stability at high temperature, and this property makes GDP-HiFi perform very well for GC-rich PCR. Being highly thermo-stable, GDP-HiFi DNA Polymerase can remain viable even after being subjected to boiling for 2 minutes. 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 PCR reactions of 50 μ l each.

Features

- 5'→3' DNA polymerase activity
- High reaction rate: 10 seconds/kb.
- Generates blunt end amplicon.
- 3'→5' exonuclease (proofreading) activity.
- High fidelity: 70 times higher than *Taq* polymerase.

Applications

- Clinical diagnosis.
- Knockout analysis.

Kit Contents

Contents	MB601-0100	MB601-0010
GDP-HiFi DNA Polymerase (1 U/ μ l)	100 μ l x 1 vial	10 μ l x 1 vial
10X GDP-HiFi PCR buffer	1 ml x 1 vial	100 μ l x 1 vial
25 mM Magnesium Sulfate	1 ml x 1 vial	100 μ l x 1 vial
dNTP Mix (2mM each)	1 ml x 1 vial	100 μ l x 1 vial
DMSO	1 ml x 1 vial	100 μ l x 1 vial

Quality Control

The quality of the GDP-HiFi DNA Polymerase is tested on a lot-to-lot basis to ensure consistent product quality.

Storage Buffer of GDP-HiFi DNA Polymerase

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 minutes at 74°C.

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
cDNA	~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 μ M 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 μ M 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 T_m (melting temperature) 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, and we 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. We recommended use up to 60 seconds per kb for maximum yield.

Required Materials

- PCR tube
- PCR instrument
- RNase-free H_2O
- DNA electrophoresis equipment

GDP-HiFi DNA Polymerase Protocol

1. For each 50 μ l reaction, assemble the following components in a 0.2 ml PCR tube on ice before the experiment:

Component	Volume (μ l)	Final Concentration
10X GDP-HiFi PCR Buffer	5 μ l	1X
2 mM dNTP Mix	5 μ l	0.2 mM each
2 mM $MgSO_4$	2 μ l	1 mM
Primer mix (10 μ M each)	1.5 μ l	0.3 μ M each
Template DNA	Variable	see Template Part
GDP-HiFi DNA Polymerase (1 U/ μ l)	1 μ l	1 U
DMSO (for GC-rich target)	Variable	5~10%
Autoclaved, distilled water to	50 μ l	

2. Mix gently. If necessary, centrifuge briefly. Cap the tube and place it in the thermal cycler.

3. To process in the thermal cycler for 35 cycles as follows:

Process	Temperature (°C)	Time	Cycles
Initial Denaturation	96-98	2 minutes	1
Denaturation	94	15 seconds	35
Annealing	60-68 (T _m of primers minus 2°C)	10-30 seconds	
Extension	68	30-60 seconds per kb of PCR product	
Final extension	68	5 minutes	1

Note: Optimal conditions for amplification will vary depending on the primers and thermal cycler used. It may be necessary to optimize the system.

4. After the PCR reaction, analyze products using standard agarose gel electrophoresis.

Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when you did PCR amplification with the kit.

Problem	Cause	Solution
Low yield of PCR products	Incomplete concentration of start materials	Use the appropriate method for the DNA preparation based on the amount of the starting materials.
DNA degrade	DNA is not fresh	Avoid repeated freeze / thaw cycles of the sample.
		Keep DNA preparations on ice or frozen in order to avoid the degradation.
	DNase contaminant	Use the fresh TAE or TBE electrophoresis buffer.
		Maintain a sterile work environment to avoid contamination from DNase.

Related Ordering Information

Cat. No.	Description	Size
AGT001-0500	AGAROSE Tablet, 0.5g	100 Tablets
BK001	BlooK LED Transilluminator	1 Set
DM010-R500	1 Kb DNA Ladder RTU	500 µl
NA005-0100	Plasmid <i>mini</i> PREP Kit	100 Reactions
LD001-1000	Novel Juice Supplied in 6X Loading Buffer	1000 µl

Caution

- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.