GDP-HiFi DNA Polymerase

Cat#: orb180490 (Protocol)

Concentration of GDP-HiFi DNA polymerase: 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 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 MgSO4 is provided. Further optimization can be achieved for different targets of DNAs. Reagents are provided for 100 PCR reactions of 50 µl each.

Features

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

Applications

Clinical diagnosis.
Knockout analysis.

Kit Contents

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

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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 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 (melting temperature) of the primers used. A range of 58-68°C is recommended. MgSO4: MgSO4 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 MgSO4 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 H2O
- DNA electrophoresis equipment

GDP-HiFi DNA Polymerase Protocol

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

Component	Volume (µl)	Final Concentration
10X GDP-HiFi PCR Buffer	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	Variable	see Template Part
GDP-HiFi DNA Polymerase (1 U/µl)	1 µl	1 U
DMSO (for GC-rich target)	Variable	5~10%
Autoclaved, distilled water to	50 µl	

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	Temperuture (°C)	Time	Cycles
Initial Denaturation	96-98	2 minutes	1
Denaturation	94	15 seconds	35
Annealing	60-68 (Tm 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.

Caution

- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- All products are for research use only.