Biozym B7 Hot-Start High Fidelity DNA Polymerase

Product information - storage

Store between -15 and -30°C.
The kit may be stored at 4°C for short-term use (up to 1 month).

Component	332040X 20 Units (2 U/µl)	332040S 100 Units (2 U/µl)	332040L 500 Units (2 U/µl)
Biozym B7 HS High Fidelity DNA Polymerase	1 x 10 µl	1 x 50 µl	1 x 50 µl
5x B7 Reaction Buffer	1 x 0.2 ml	1 x 1.7 ml	3 x 1.7 ml
10x B7 Enhancer	1 x 0.3 ml	1 x 1.7 ml	2 x 1.7 ml

For research use only.

1. Introduction

Biozym B7 Hot-Start High Fidelity DNA Polymerase is designed for applications where sequence accuracy is a key demand. The enzyme is ideal for sequencing, site-directed mutagenesis and cloning applications. Derived from *Pfu* DNA polymerase the enzyme shows 3'-5' exonuclease (proofreading) activity. The error rate of the Biozym B7 Hot-Start High Fidelity DNA Polymerase is about 100 times less than *Taq* DNA polymerase.
Increased processivity enables shorter extension times and higher yield - even with challenging and longer templates.

Using latest hot-start technology inhibits DNA polymerase activity at ambient temperature, thus preventing primer dimer formation and amplification of non-specific products. Reaction set-up can be performed at room temperature.

2. Notes

- Biozym B7 Hot Start High-Fidelity DNA Polymerase produces blunt end amplicons
- Fidelity: Approximately 100 times higher than *Taq* DNA polymerase
- Amplicon lengths: Up to 20 kb
- Hot-start technology enables set up at room temperature

3. Technical support

For technical support please contact support@biozym.com

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4. Reaction conditions for PCR

Carefully mix and centrifuge the tube before opening to ensure homogeneity and improve recovery.

Table 1. Pipetting instructions

Component	50 µl reaction	20 µl reaction	Final conc.	Notes
5x B7 Reaction Buffer	10 µl	4 µl	1x	
B7 HS High Fidelity DNA Polymerase	0.5 µl	0.2 µl		
Forward primer (10 µM)	2.0 µl	0.8 µl	400 nM	See 5.2 for primer design
Reverse primer (10 µM)	2.0 µl	0.8 µl	400 nM	
Template DNA Less complex DNA Genomic DNA	 <10 ng <0.2 µg	 <4 ng <0.1 µg	Variable	
Optional: 10x B7 Enhancer	5 µl	2 µl	1x	See 5.3 for enhancer
PCR grade water	Up to 50 µl reaction volume	Up to 20 µl reaction volume		

Table 2. Cycling instructions

Cycles	Temperature	Time	Notes
1	95°C	60 seconds	Initial template denaturation
25 - 40	95°C 60 to 75°C 72°C	15 seconds 15 seconds 30 seconds/kb	Denaturation Annealing Extension

5. Notes about reaction components and cycling conditions

5.1. Reaction Buffer

The 5x B7 reaction buffer contains: 15 mM $MgCl_2$, 5 mM dNTPs, enhancers and stabilizers. We do not recommend adding further separate PCR enhancers (exception please see 5.3) or $MgCl_2$.

5.2. Primers

Primers should have a predicted melting temperature of around 60°C, using default Primer 3 settings (https://bioinfo.ut.ee/primer3/). The final primer concentration in the reaction should be between 0.2 µM and 0.6 µM.

5.3. 10x Enhancer

Long templates, GC-rich or templates with complex secondary structure: In case of no or weak amplification addition of 10x B7 Enhancer can improve yield.

5.4. Annealing

Use an annealing temperature equal to the Tm of the lower Tm primer. If non-specific products are present increase in 2°C increments. Alternatively use a temperature gradient to experimentally find the optimal annealing temperature.

5.5. Extension

Extension should be performed at 72°C. The optimal extension time is dependent on amplicon length and complexity of template. We recommend an extension time of 30 seconds per kilobase (kb) for most templates. In case of 2-step protocols 68 to 75°C could be used as combines annealing/extension temperature.

5.6. Multiplex PCR

When first performing multiplex PCR it is recommended to run a temperature gradient around the calculated annealing temperatures. The annealing temperature representing best specificity should be used in subsequent experiments. Fast cycling conditions should not be used. Initially using of the extension time of the longest fragment is recommended.