Herculase Enhanced DNA Polymerase

Instruction Manual

Catalog #600260 (100 U), #600262 (500 U), #600264 (1000 U), and #600266 (5000 U)
Revision E0

Laboratory Reagent.
600260-12

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MATERIALS PROVIDED

Materials provided	Quantity			
	Catalog #600260 (100 U)	**Catalog #600262 (500 U)**	**Catalog #600264 (1000 U)**	**Catalog #600266 (5000 U)**
Herculase enhanced DNA polymerase (5 U/ µl)	20 µl	100 µl	200 µl	5 × 200 µl
10× Herculase reaction buffer	1 ml	2 × 1 ml	4 × 1 ml	20 × 1 ml
Dimethyl Sulfoxide (DMSO)	1 ml	1 ml	1 ml	5 × 1 ml

STORAGE CONDITIONS

All Components: –20°C

NOTICES TO PURCHASER

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Revision E0

INTRODUCTION

Herculase enhanced DNA polymerase provides superior performance in applications requiring high fidelity amplification over a broad range of target lengths and complexities (0.1–48 kb).[1] Herculase polymerase features a novel DNA polymerase composition that consists predominantly of *Pfu* DNA polymerase, combined with the exclusive thermostable ArchaeMaxx polymerase-enhancing factor and a small amount of *Taq2000* DNA polymerase. Our unique *Pfu*-based formulation is provided with a buffer optimized to promote high yield, specificity, and amplification of extra long targets. As a result, the Herculase enhanced DNA polymerase can be used to successfully amplify small targets as well as genomic targets up to 37 kb and vector targets up to 48 kb, while maintaining a lower error rate than other DNA polymerase mixtures.

A key component of Herculase enhanced DNA polymerase is the ArchaeMaxx polymerase-enhancing factor. The ArchaeMaxx factor eliminates a PCR inhibitor and promotes shorter extension times, higher yield, and greater target length capabilities. The ArchaeMaxx factor improves the yield of products by overcoming dUTP poisoning, which is caused by dUTP accumulation during PCR through dCTP deamination.[2] Once incorporated, dU-containing DNA inhibits *Pfu* and most archaeal proofreading DNA polymerases, such as Vent and Deep Vent DNA polymerases, limiting their efficiency.[2] The ArchaeMaxx factor functions as a dUTPase, converting poisonous dUTP to harmless dUMP and inorganic pyrophosphate, resulting in improved overall PCR performance.

CRITICAL OPTIMIZATION PARAMETERS

All PCR amplification reactions require optimization to achieve the highest product yield and specificity. Critical optimization parameters for successful PCR using Herculase DNA polymerase are outlined in Table I and are discussed in the following section. The provided Herculase buffer contains the magnesium ion concentration that is optimal for the enzyme. Adjusting the magnesium concentration is not recommended.

TABLE I

Optimization Parameters and Suggested Reaction Conditions (50 µl reaction volume)

Parameter	Typical Targets ≤10 kb	G-C Rich Targets <10 kb	Targets >10 kb
Input template	100–200 ng genomic DNA 1–15 ng vector DNA	100–200 ng genomic DNA 1–15 ng vector DNA	250–1000 ng genomic DNA 15–60 ng vector DNA
Herculase Enhanced DNA polymerase	2.5 U	2.5 U	5.0 U
DMSO concentration[a]	0%	4–8%	0–3% for genomic targets <23 kb 3–6% for genomic targets >23 kb 5–7% for lambda targets >30 kb 4–8% for G-C rich targets
Primers (each)	~100 ng (0.25 µM)	~100 ng (0.25 µM)	~200 ng (0.5 µM)
dNTP concentration	200 µM each dNTP (0.8 mM total)	200 µM each dNTP (0.8 mM total)	500 µM each dNTP (2 mM total)
Extension time	1 min per kb	1 min per kb	≥1 min per kb, not to exceed 1 hour
Extension temperature	72°C	72°C	68°C
Denaturing temperature	92–95°C	92–98°C	92°C

[a] We suggest titration of DMSO in 1% increments over the indicated range for each set of templates/primer pairs.

DNA Template Quality and Concentration

Successful amplification is dependent upon the purity, integrity, concentration, and molecular weight of the DNA template. Isolation of intact, high molecular weight genomic DNA may be achieved by using the Agilent DNA Extraction Kit or the RecoverEase DNA isolation kit. Potential shearing of the genomic DNA template is minimized by the use of wide-bore tips for pipetting or mixing of the template. Additionally, freezing of high molecular weight templates should be avoided; storage at 4°C is recommended. The length of an intact genomic DNA template should be >50 kb.

For amplifying genomic DNA templates, we recommend using 100–250 ng of template for targets of ≤10 kb. Optimal concentrations of template for longer complex targets, up to 37 kb, may range between 250 ng and 1 µg using reaction volumes of 50 µl. To amplify low-complexity targets (for example, lambda DNA or cloned DNA), we recommend using 1–15 ng for targets ≤10 kb and 15–60 ng for targets >10 kb in a 50-µl reaction volume. Excess template DNA can inhibit the PCR reaction.

Enzyme Concentration

Robust product yield requires an adequate DNA polymerase concentration. The use of 2.5 U/50-µl reaction consistently generates high yield of templates ≤10 kb. Longer templates require 5 U of Herculase DNA polymerase per 50-µl reaction for optimal results.

DMSO

DMSO is provided as a means of obtaining higher yields of PCR product with extra-long targets or GC-rich targets. The DMSO concentration must be titrated for each application, since the degree to which DMSO enhances product yield and specificity varies according to target length, complexity, and GC content.

For genomic DNA, begin with the optimization guidelines of 0–3% DMSO for 10–23 kb targets, and 3–6% DMSO for targets >23 kb. For lambda DNA targets >30 kb, begin optimization using 5–7% DMSO. For GC-rich targets, DMSO at 4–8% is generally recommended. The DMSO concentration should be titrated in the specified range in 1% increments.

Note *The addition of DMSO may increase the error rate of the Herculase polymerase slightly (<50% increase with 3% DMSO). The use of DMSO is discouraged when the highest fidelity is essential.*

Primer Design and Concentration

Primers should be ≥23 bp in length with a balanced T_m ≥60°C. The resulting high annealing temperature promotes specificity and discourages secondary structure formation. Further, primer sequences should be analyzed for potential duplex and hairpin formation as well as false priming sites in order to obtain the highest yield of specific PCR products.

We suggest using ~0.25 µM final concentration of each primer for targets <10 kb, .and ~0.5 µM final concentration of each primer for targets >10 kb. When using 25-mer oligonucleotide primers in a 50-µl reaction volume, this is equivalent to ~100 ng or ~200 ng, respectively, of each primer.

Deoxynucleotide Concentrations

Amplification efficiencies are influenced by deoxynucleotide (dNTP) concentrations. Insufficient concentrations of dNTPs may result in lower yields. For targets ≤10 kb, 200 µM each dNTP is recommended; for targets ≥10 kb, the use of 500 µM each dNTP is optimal.

Cycling Parameters

As with all PCR reactions, cycling parameters are critical for successful amplification and may require further optimization.

Extension Time

Use an extension time of 1.0 minute/kb of template for general applications; longer extension times may produce higher yields, however, an extension time exceeding one hour provides no further benefit.

Extension Temperature

Extension temperatures also have a critical effect on amplicon yield. An extension temperature of 72°C should be used with templates less than 10 kb, while templates greater than 10 kb in length require an extension temperature of 68°C.

Denaturation Temperature

High denaturation temperatures damage DNA templates, so the denaturation temperature should be as low as possible. A denaturation temperature of 92°C works well for most targets. For GC-rich targets, which are difficult to melt, a denaturation temperature of 98°C is recommended.

GC-Rich Targets

For amplification of GC-rich targets, we recommend including DMSO in the reaction at a concentration of 4–8% and using a PCR program with a denaturation temperature of 98°C and an extension temperature of 72°C.

PROTOCOL

1. Prepare a reaction mixture for the appropriate number of samples to be amplified. The following table provides an example of a reaction mixture for the amplification of targets ≤10 kb, targets >10 kb, and GC-rich targets (which are typically ≤10 kb). The recipe listed in the table is for one reaction and can be adjusted for multiple samples. Add the components *in order* and mix gently.

Component	Quantity per reaction		
	≤10-kb targets	**>10-kb targets**	**GC-rich targets ≤10 kb**
Distilled water	X µl to final volume of 50.0 µl	X µl to final volume of 50.0 µl	X µl to final volume of 50.0 µl
10×Herculase reaction buffer	5.0 µl	5.0 µl	5.0 µl
dNTP mix (25 mM of each dNTP)	0.4 µl	1.0 µl	0.4 µl
DNA template: Genomic DNA Low-complexity templates (λ DNA or cloned DNA)	 100–250 ng 1–15 ng	 250–1000 ng 15–60 ng	 100–250 ng 1–15 ng
Primer #1	100 ng	200 ng	100 ng
Primer #2	100 ng	200 ng	100 ng
Herculase polymerase (5 U/µl)	0.5 µl	1.0 µl	0.5 µl
DMSO[a]	—	Either 0–3%, 3–6%, 5–7% or 4–8%	4–8%
Total reaction volume	50.0 µl	50.0 µl	50.0 µl

[a] The optimal DMSO concentration must be determined by titration in 1% increments for each primer-template set. See *Critical Optimization Parameters* for DMSO concentration range recommendations for specific targets.

2. Before thermal cycling, aliquot 50 µl of the master mixture into sterile thin-walled PCR tubes.

3. Perform PCR using optimized cycling conditions. Suggested cycling parameters are given below for (A) targets with average base composistion; and (B) GC-rich targets.

(A)

Average Base Composition Targets (>10 kb or ≤10 kb)

Segment	Number of cycles	Temperature		Duration	
		Targets >10 kb	Targets ≤10 kb	Targets >10 kb	Targets ≤10 kb
1	1	92°C	95°C	2 minutes	2 minutes
2	10	92°C	95°C	10 seconds	30 seconds
		Primer T_m – 5°C[a]	Primer T_m – 5°C[a]	30 seconds	30 seconds
		68°C	72°C	60 seconds/kb of PCR target	60 seconds/kb of PCR target
3	20	92°C	95°C	10 seconds	30 seconds
		Primer T_m – 5°C	Primer T_m – 5°C	30 seconds	30 seconds
		68°C	72°C	60 seconds/kb of PCR target plus 10 seconds/cycle	60 seconds/kb of PCR target plus 10 seconds/cycle

[a] The annealing temperature may be lowered or raised further if necessary to obtain optimal results. Typical annealing temperatures will range between 60 and 65°C.

(B)

GC-Rich Targets (≤10 kb)

Segment	Number of cycles	Temperature	Duration
1	1	98°C	3 minutes
2	10	98°C	40 seconds
		Primer T_m – 5°C	30 seconds
		72°C	60 seconds/kb of PCR target[a]
3	20–25	98°C	40 seconds
		Primer T_m – 5°C	30 seconds
		72°C	60 seconds/kb of PCR target plus 10 seconds/cycle[a]
4	1	72°C	10 minutes

[a] 1 minute minimum extension.

4. Analyze the PCR amplification products by electrophoresis using an appropriate percentage acrylamide or agarose gel. Long PCR products greater than 17 kb in length may be separated on a 0.6% agarose gel, however a 0.8% agarose gel may be used if higher resolution with less separation is desired. For maximum separation and resolution, pulse field gel electrophoresis with a 1.0% gel is recommended.

TROUBLESHOOTING

Observations	Suggestions
No PCR product or lower yield than expected	Increase the amount of Herculase polymerase (up to 10 U can be used for targets >23 kb)
	Increase the amount of full-length intact DNA template and/or increase the number of cycles up to a maximum of 40 cycles
	Use intact and highly purified DNA templates
	Store the template at 4°C; do not freeze the template
	Lower the annealing temperature in 5°C increments
	Allow at least 60 seconds of extension time for each kilobase to be amplified (90 seconds of extension time per kilobase may also be helpful for difficult templates). For GC-rich targets, use a minimum extension time of 1 minute
	For targets >10 kb, denaturation at 92°C for 10 seconds is usually sufficient. Longer denaturation times or higher denaturation temperatures may damage the DNA template; use the shortest denaturation time compatible with successful PCR on the thermal cycler For targets ≤10 kb, denaturation at 95°C for 30 seconds is usually sufficient. For GC-rich targets, denaturation at 98°C for 40 seconds is usually sufficient.
	Primer pairs exhibiting matched primer melting temperatures (T_m) and complete complementarity to the template are recommended
	Analyze the primers to ensure that duplexes or hairpins do not form
	Gel-purified or HPLC-purified primers ≥23 nucleotides in length are recommended
	Purify the primers by PAGE or HPLC
	Adjust the ratio of primer versus template to optimize yield of the desired product
	Use DMSO in the PCR mixture; titrate the DMSO concentration in 1% increments
Artifactual PCR smears	Decrease the amount of Herculase polymerase
	Reduce the extension time
	Optimize the cycling parameters specifically for the primer–template set and the thermal cycler used
Multiple bands	Increase the annealing temperature in 5°C increments
	Use Perfect Match PCR enhancer to improve PCR product specificity
	Use DMSO in the PCR mixture;titrate the DMSO concentration
	Verify that the primers hybridize only to the desired sequences on the template

REFERENCES

1. Borns, M. and Hogrefe, H. H. (2000) *Strategies* 13(1):1-3.
2. Hogrefe, H. H., Hansen, C. J., Scott, B. R. and Nielson, K. B. (2002) *Proc Natl Acad Sci U S A* 99(2):596-601.

MSDS INFORMATION

Material Safety Data Sheets (MSDSs) are provided online at *www.agilent.com*. MSDS documents are not included with product shipments.

Herculase Enhanced DNA Polymerase

Catalog #600260, #600262, #600264, and #600266

QUICK-REFERENCE PROTOCOL (TEMPLATES ≤10 KB)*

Prepare reaction mixtures according to the table below, mix gently, and place in thin-walled PCR tubes.

Component	Quantity per reaction	
	Average composition targets	**GC-rich targets**
Distilled water	X µl to final volume of 50.0 µl	X µl to final volume of 50.0 µl
10×Herculase reaction buffer	5.0 µl	5.0 µl
dNTP mix (25 mM of each dNTP)	0.4 µl	0.4 µl
DNA template:		
Genomic DNA	100–250 ng	100–250 ng
Low-complexity templates	1–15 ng	1–15 ng
Primer #1	100 ng	100 ng
Primer #2	100 ng	100 ng
Herculase hotstart polymerase (5 U/µl)	0.5 µl	0.5 µl
DMSO	—	4–8%
Total reaction volume	50.0 µl	50.0 µl

Perform PCR using the cycling conditions appropriate for your target base composition, according to the following tables:

(A) Single block temperature cyclers (average base composition targets)
(B) Single block temperature cyclers (GC-rich targets)

* For targets >10 kb, see *Protocol* section of manual.

(A) Average Base Composition Targets ≤10 kb

Segment	Number of cycles	Temperature	Duration
1	1	95°C	2 minutes
2	10	95°C	30 seconds
		PrimerT_m – 5°C	30 seconds
		72°C	60 seconds/kb target
3	20	95°C	30 seconds
		PrimerT_m – 5°C	30 seconds
		72°C	60 seconds/kb target plus 10 seconds per cycle

(B) GC-Rich Targets ≤10 kb

Segment	Number of cycles	Temperature	Duration
1	1	98°C	3 minutes
2	10	98°C	40 seconds
		PrimerT_m – 5°C	30 seconds
		72°C	60 seconds/kb target
3	20–25	98°C	40 seconds
		PrimerT_m – 5°C	30 seconds
		72°C	60 seconds/kb target plus 10 seconds per cycle
4	1	72°C	10 minutes

Analyze the PCR amplification products by gel electrophoresis