Herculase Hotstart DNA Polymerase

INSTRUCTION MANUAL

Catalog #600310 (100 U), #600312 (500 U), and #600314 (1000 U)
Revision A.01

For In Vitro Use Only
600310-12

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Herculase Hotstart DNA Polymerase

MATERIALS PROVIDED

Material provided	Quantity		
	Catalog #600310 (100 U)	Catalog #600312 (500 U)	Catalog #600314 (1000 U)
Herculase hotstart DNA polymerase (5 U/ µl)	20 µl	100 µl	200 µl
10× Herculase reaction buffer	1 ml	2 × 1 ml	4 × 1 ml
Dimethyl Sulfoxide (DMSO)	1 ml	1 ml	1 ml

STORAGE CONDITIONS

All Components: –20°C

NOTICES TO PURCHASER

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This product is covered by the claims of one or more of the following U.S. Patents: 5,545,552; 5,556,772; 5,866,395; 5,948,663; 6,489,150; 6,183,997; 6,333,165; 6,379,553; 6,444,428. Purchase of this product conveys to the purchaser only the non-transferable right under these patents to use the product ***for research use only by the purchaser***. No rights are granted to the purchaser hereunder to sell, modify for resale or otherwise transfer this product, either alone or as a component of another product, to any third party. Agilent reserves all other rights, and this product may not be used in any manner other than as provided herein. For information on obtaining a license to use this product for purposes other than research, please contact the Stratagene Products Division, Business Development, 11011 North Torrey Pines Road, La Jolla, California 92037. Phone (858) 535-5400.

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Revision A.01

INTRODUCTION

Herculase hotstart 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, 2] Herculase hotstart DNA polymerase features a novel DNA polymerase composition that consists predominantly of *Pfu* DNA polymerase, combined the exclusive thermostable ArchaeMaxx polymerase-enhancing factor and *Taq2000* DNA polymerase. This unique *Pfu*-based formulation is provided with a buffer optimized to promote high yield, specificity, and amplification of a wide range of targets. As a result, Herculase hotstart 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 an accuracy greater than *Taq* DNA polymerase and other DNA polymerase mixtures.

A key component of Herculase hotstart 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.[3] Once incorporated, dU-containing DNA inhibits *Pfu* and most archaeal proofreading DNA polymerases, such as Vent® and Deep Vent® DNA polymerases, limiting their efficiency.[3] The ArchaeMaxx factor functions as a dUTPase, converting poisonous dUTP to harmless dUMP and inorganic pyrophosphate, resulting in improved overall PCR performance.

Herculase hotstart DNA polymerase provides high specificity and detection sensitivity, while facilitating high-throughput robotic PCR applications. Herculase hotstart DNA polymerase is formulated with a combination of antibodies that effectively neutralize DNA polymerase and 3´-5´ exonuclease (proofreading) activities. Full enzyme activity is regained upon denaturation of the antibodies during the initial denaturation step. See the table in *Properties of Stratagene Hot-Start PCR Enzymes*, below, for a comparison of the Stratagene Hot-Start PCR enzymes.

* U.S. Patent Nos. 6,734,293, 6,489,150; 6,444,428; 6,183,997; 5,948,663; 5,866,395; 5,556,772; 5,545,552 and patents pending.

PROPERTIES OF STRATAGENE HOT-START PCR ENZYMES

Hot Start PCR enzyme	Hot Start Method	Activities Neutralized	Activation Procedure[a]	Applications
Herculase hotstart DNA polymerase	Antibody	DNA polymerase, 3′-5′ exonuclease	PCR Activation 30 cycles	• challenging cloning targets • long and/or GC-rich targets • higher fidelity than *Taq* DNA polymerase
PfuTurbo hotstart DNA polymerase	Antibody	DNA polymerase, 3′-5′ exonuclease	PCR Activation 30 cycles	• Highest fidelity • Genomic DNA templates up to 19 kb
SureStart *Taq* DNA polymerase	Chemical	DNA polymerase, 5′-3′ exonuclease	Pre-PCR Activation (9–12 minutes @ 95°C) 30 cycles or PCR Activation 40 cycles	• Routine PCR up to 3 kb
YieldAce hotstart DNA polymerase	Antibody	DNA polymerase	PCR Activation 30 cycles	• maximum target yields • superior yield compared to *Taq* DNA polymerase • amplification of clones for microarrays

[a] PCR activation means that full enzyme activity is recovered during temperature cycling, either during the initial denaturation step (antibody-based formulations) or within the first 5–15 cycles (chemical hot start). For SureStart *Taq* DNA polymerase, slow enzyme activation during temperature cycling typically necessitates the use of additional PCR cycles to achieve desired product yield (35–45 cycles). In the Pre-PCR activation method, the enzyme is activated prior to temperature cycling, and no additional cycles are necessary.

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 hotstart DNA polymerase are outlined in Table I and discussed in the following section. The provided Herculase hotstart 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 Hotstart 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] Titrate the 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 Stratagene 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, use 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), use 1–15 ng for targets ≤10 kb and 15–60 ng for targets >10 kb. 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 hotstart 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.

Use ~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

Maintain 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, and is sufficient to denature the hotstart antibodies. 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 using 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 hotstart 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. If the temperature-cycler is not equipped with a heated cover, overlay each reaction with ~50 µl of DNase-, RNase-, and protease-free mineral oil (available from Sigma Chemical Company, St. Louis, Missouri). If the extension times are >15 minutes, overlay each reaction with mineral oil even if the temperature-cycler is equipped with a heated cover.

4. Perform PCR using optimized cycling conditions. Suggested cycling parameters are given below for (A) single-block temperature cyclers, (B) single-block temperature cyclers with GC-rich targets, (C) RoboCycler temperature cyclers and (D) RoboCycler temperature cyclers with GC-rich targets:

(A)

Single-Block Temperature Cyclers

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)

Single-Block Temperature Cyclers with 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.

(C)

RoboCycler Temperature Cyclers

Segment	Number of cycles	Temperature	Duration
1	1	92°C	2 minutes
2	30–35	92°C	30 seconds
		Primer T_m – 5°C [a]	30 seconds
		68°C [b]	60 seconds/kb of PCR target
3	1	68°C [b]	10 minutes

[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] Templates >10 kb in length require an extension temperature of 68°C. An extension temperature of 72°C should be used with templates ≤10 kb.

(D)

RoboCycler Temperature Cyclers with GC-Rich Targets ≤10 kb

Segment	Number of cycles	Temperature	Duration
1	1	98°C	3 minutes
2	30–35	98°C	1 minute
		Primer T_m – 5°C	40 seconds
		72°C	60 seconds/kb of PCR target[a]
3	1	72°C	10 minutes

[a] 1 minute minimum extension.

5. 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 hotstart polymerase (up to 10 U can be used for targets >23 kb)
	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 times of 30 seconds for RoboCycler temperature cyclers or 10 seconds for single-block temperature cyclers at 92°C are usually sufficient, while 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 times of 1 minute for RoboCycler temperature cyclers or 30 seconds for single-block temperature cyclers at 95°C are usually sufficient. For GC-rich targets, denaturation times of 1 minute for RoboCycler temperature cyclers or 40 seconds for single-block temperature cyclers at 98°C are 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 thin-wall PCR tubes for Stratagene RoboCycler thermal cyclers. These PCR tubes are optimized to ensure ideal contact with the multiblock design to permit more efficient heat transfer and to maximize thermal-cycling performance
	For targets >10 kb, increase the amount of template DNA
Artifactual PCR smears	Use DMSO in the PCR mixture; titrate the DMSO concentration in 1% increments
	Decrease the amount of Herculase hotstart polymerase
	Reduce the extension time
Multiple bands	Optimize the cycling parameters specifically for the primer–template set and the thermal cycler used
	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. Borns, M. and Hogrefe, H. H. (2001) *Strategies* 14(2):41–42.
3. 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.

ENDNOTES

MSDS INFORMATION

The Material Safety Data Sheet (MSDS) information for Stratagene products is provided on the web at *http://www.stratagene.com/MSDS/*. Simply enter the catalog number to retrieve any associated MSDS's in a print-ready format. MSDS documents are not included with product shipments.

STRATAGENE
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Herculase Hotstart DNA Polymerase

Catalog #600310, 600312, 600314

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 Low-complexity templates	 100–250 ng 1–15 ng	 100–250 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

- If the temperature cycler is not equipped with a heated cover, or if extension times are >15 minutes, overlay each reaction mixture with 50 µl mineral oil.

- Perform PCR using the cycling conditions appropriate for your temperature cycler and 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)
  (C) RoboCycler temperature cyclers (average or GC-rich targets)

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

(A) Single-Block Temperature Cyclers, 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) Single-Block Temperature Cyclers, 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

(C) RoboCycler Temperature Cyclers, Targets ≤10 kb

Segment	Number of cycles	Average Base Composition Targets		GC-Rich Targets	
		Temperature	Duration	Temperature	Duration
1	1	92°C	2 minutes	98°C	3 minutes
2	30–35	92°C	30 seconds	98°C	1 minute
		Primer T_m – 5°C	30 seconds	Primer T_m – 5°C	40 seconds
		68°C	1 minute/kb target	72°C	1 minute/kb target
3	1	68°C	10 minutes	72°C	10 minutes

- Analyze the PCR amplification products by gel electrophoresis