Herculase II Fusion Enzyme with dNTPs Combo

Catalog #600677, 600679

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Materials provided	Quantity	
	Catalog #600677	Catalog #600679
Herculase II Fusion DNA Polymerase	200 µl	400 µl
5× Herculase II Reaction Buffer	2 × 1.5 ml	4 × 1.5 ml
Dimethylsulfoxide (DMSO)	1 ml	1 ml
dNTPs (100 mM; 25 mM each dNTP)	100 µl	200 µl

Storage: Store at –20°C upon receipt.

Introduction

In Agilent's Herculase II fusion DNA polymerase,* we have dramatically increased processivity of high-fidelity PCR by fusing our *Pfu*-based DNA polymerase** with a high affinity double-stranded DNA binding domain. Enhanced processivity and the inclusion of our exclusive ArchaeMaxx PCR enhancing factor make it ideal for routine PCR applications that demand superior yield and excellent reliability, with shorter cycling times. Herculase II fusion DNA polymerase provides accuracy comparable to *Pfu* DNA polymerase. Moreover, the special enzyme formulation and optimized buffer system ensure robust performance when amplifying difficult and GC-rich targets. Using the modified cycling protocol described here, Herculase II fusion DNA polymerase also provides excellent performance for long-range PCR of targets >10 kb.

Optimization Parameters (50-µL reaction volume)

Parameter	Targets <1 kb	Targets 1–10 kb	Targets >10 kb	cDNA Targets
Input template DNA	100–300 ng genomic DNA or 1–30 ng vector DNA	100–400 ng genomic DNA or 1–30 ng vector DNA	150–400 ng genomic DNA or 15–60 ng vector DNA	1–2 µl cDNA from RT-PCR reaction
Herculase II fusion DNA polymerase	0.5 µl	1 µl	1 µl	1 µl
DMSO	0–8% final concentration[a]	0–8% final concentration[a]	0–8% final concentration[a]	0–8% final concentration[a]
Primers (each)	0.25 µM	0.25 µM	0.5 µM	0.25 µM
dNTPs	250 µM each dNTP	250 µM each dNTP	250 µM each dNTP	400 µM each dNTP
Extension time	30 seconds	30 seconds per kb	Use incremental cycling protocol (see *Cycling Parameters* table)	60 seconds per kb
Denaturing temp	95°C[b]	95°C[b]	92°C	95°C
Extension temp	72°C	72°C	68°C	68°C

[a] Titrate DMSO in 1% increments. For targets with typical base composition, if target<20 kb titrate at 0–3%, or if target>20 kb titrate at 0–6%. For GC-rich targets, titrate at 0–8%. DMSO may increase PCR error rates slightly so should be avoided in cases where there is no benefit to yield or specificity.

[b] For GC-rich targets, increase the denaturing temperature to 98°C.

PCR Protocol

The reaction conditions given here are for one reaction and must be adjusted for multiple samples. The final volume of each sample reaction is 50 µl. Add the components in order into sterile thin-walled PCR tubes while mixing gently.

Component	Quantity per reaction			
	Targets <1 kb	Targets 1–10 kb	Targets >10 kb	cDNA Targets
Distilled water (dH_2O)	X µl to final 50 µl volume	X µl to final 50 µl volume	X µl to final 50 µl volume	X µl to final 50 µl volume
5× Herculase II reaction buffer[a]	10 µl	10 µl	10 µl	10 µl
dNTPs (25 mM each)	0.5 µl	0.5 µl	0.5 µl	0.8 µl
DNA template [b]	X µl (see *Optimization Parameters*)	X µl (see *Optimization Parameters*)	X µl (see *Optimization Parameters*)	1–2 µl cDNA from RT-PCR reaction
Primer #1 (10 µM) [c]	1.25 µl	1.25 µl	2.5 µl	1.25 µl
Primer #2 (10 µM) [c]	1.25 µl	1.25 µl	2.5 µl	1.25 µl
Herculase II fusion DNA polymerase	0.5 µl	1.0 µl	1.0 µl	1.0 µl
DMSO [d]	X µl (titrate to optimize)	X µl (titrate to optimize)	X µl (titrate to optimize)	X µl (titrate to optimize)
Total reaction volume	50.0 µl	50.0 µl	50.0 µl	50.0 µl

[a] The 5× buffer provides a final 1× Mg^{2+} concentration of 2 mM.

[b] The amount of DNA template required varies depending on the type of DNA being amplified (see *Optimization Parameters* for guidelines). Successful amplification of long targets is especially dependent on genomic DNA purity, integrity and molecular weight (>50 kb is optimal).

[c] Yield may be improved by adjusting the ratio of primer to template. The optimal concentration provided here is for a typical 25-base oligonucleotide, where the addition of 1.25 µl of a 10 µM primer stock is equivalent to approximately 100 ng of each primer per 50-µl reaction.

[d] The optimal DMSO concentration must be determined by titration in 1% increments for each primer-template set; see *Optimization Parameters*.

Perform PCR using optimized cycling conditions. Suggested PCR cycling parameters shown below have been tested on a variety of thermal cyclers, including the Agilent Mx3000P and Mx3005P QPCR systems. Optimized cycling parameters are not necessarily transferable between thermal cyclers. Analyze the PCR amplification products on a 0.7-1.0% (w/v) agarose gel.

Cycling Parameters for Targets ≤10 kb

Segment	Number of cycles	Temperature		Duration	
		Genomic or Vector DNA Targets	cDNA Targets	Genomic or Vector DNA Targets	cDNA Targets
1	1	95°C[a]	95°C	2 minutes[a]	1 minute
2	30	95°C[a]	95°C	20 seconds	20 seconds
		Primer Tm – 5°C[b]	Primer Tm – 5°C[b]	20 seconds	20 seconds
		72°C	68°C	30 seconds for targets < 1kb or 30 seconds per kb for ≥1kb	60 seconds for targets < 1kb or 60 seconds per kb for ≥1kb
3	1	72°C	68°C	3 minutes	4 minutes

Cycling Parameters for Targets >10 kb

Segment	Number of cycles	Temperature	Duration
1	1	92°C	2 minutes
2	10	92°C	20 seconds
		Primer Tm – 5°C[b]	20 seconds
		68°C	30 seconds per kb
3	20	92°C	20 seconds
		Primer Tm – 5°C[b]	20 seconds
		68°C	Increase extension time incrementally: 30 seconds per kb + 20 seconds per cycle [c]
4	1	68°C	8 minutes

[a] When amplifying GC-rich targets ≤10 kb, increase the denaturing temperature to 98°C and the initial denaturing duration to 2–4 minutes.

[b] 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.

[c] For cycles 11–30, incrementally add 20 seconds to the total extension time of the previous cycle. For example, for cycle 11 use duration of 30 sec/kb +20 seconds; for cycle 12 use duration of 30 sec/kb +40 seconds, and so on.

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Endnotes

* U.S. Patent Nos. 6,734,293, 6,444,428, 6,183,997, 5,948,663 and 5,545,552.

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MSDS Information

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