Herculase II Fusion DNA Polymerase

Catalog #600675
Laboratory Reagent.

Technical Services
US and Canada: Call (800) 227-9770 (option 3, 4, 3).
Or send an e-mail to genomics@agilent.com
Agilent's world-wide Sales and Support contact details can be obtained at www.agilent.com/en/contact-us/page.

MATERIALS PROVIDED

Materials Provided	Quantity
Herculase II Fusion DNA Polymerase	40 µl
5× Herculase II Reaction Buffer	1.5 ml
Dimethylsulfoxide (DMSO)	1 ml

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, or to 99.9°C if using the Agilent SureCycler 8800 thermal cycler.

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.

	Quantity per reaction			
Component	**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
dNTP mix (25 mM each dNTP)	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. Refer to the tables below for suggested cycling conditions based on target length and the type of template DNA. These protocols have been tested on a variety of thermal cyclers including the Agilent SureCycler 8800. Optimized cycling parameters are not necessarily transferrable between thermal cyclers.

Cycling Conditions for Genomic or Vector DNA Targets ≤10 kb

Segment	Number of cycles	Temperature	Duration
1	1	95°C [a]	2 minutes [a]
2	30	95°C [a]	10–20 seconds [b]
		Primer Tm – 5°C [c]	20 seconds
		72°C	30 seconds for targets <1 kb or 30 seconds per kb for ≥1 kb
3	1	72°C	3 minutes

[a] When amplifying GC-rich targets on the SureCycler 8800, increase the denaturing temperature to 99.9°C. For all other cyclers, increase the denaturing temperature to 98°C and increase the initial denaturing duration to 2–4 minutes.
[b] If using the SureCycler 8800, use a 10-second denaturation during cycling. For all other cyclers, use a 20-second denaturation during cycling.
[c] Lower or raise the annealing temperature as necessary to obtain optimal results. Typical annealing temperatures will range between 60 and 65°C.

Cycling Conditions for Genomic or Vector DNA Targets >10 kb

Segment	Number of cycles	Temperature	Duration
1	1	92°–95°C [a]	2 minutes
2	10	92°–95°C [a]	10–20 seconds [b]
		Primer Tm – 5°C [c]	20 seconds
		68°C	30 seconds per kb
3	20	92°–95°C [a]	10–20 seconds [b]
		Primer Tm – 5°C [c]	20 seconds
		68°C	Increase extension time incrementally: 30 seconds per kb + 20 seconds per cycle [d]
4	1	68°C	8 minutes

[a] If using the SureCycler 8800, use 95°C for all denaturation steps. For all other cyclers,use a denaturation temperature of 92°C.
[b] If using the SureCycler 8800, use a 10-second denaturation during cycling. For all other cyclers, use a 20-second denaturation during cycling.
[c] Lower or raise the annealing temperature as necessary to obtain optimal results. Typical annealing temperatures will range between 60 and 65°C.
[d] 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.

Cycling Conditions for cDNA Targets ≤10 kb

Segment	Number of cycles	Temperature	Duration
1	1	95°C	1 minute
2	30	95°C	10–20 seconds [a]
		Primer Tm – 5°C [b]	20 seconds
		68°C	60 seconds for targets < 1kb or 60 seconds per kb for ≥1kb
3	1	68°C	4 minutes

[a] If using the SureCycler 8800, use a 10-second denaturation during cycling. For all other cyclers, use a 20-second denaturation during cycling.
[b] Lower or raise the annealing temperature as necessary to obtain optimal results. Typical annealing temperatures will range between 60 and 65°C.

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MSDS INFORMATION

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