EXL DNA Polymerase

INSTRUCTION MANUAL

Catalog #600340 (100 U), #600342 (500 U), and #600344 (1000 U)
Revision A.01

For In Vitro Use Only
600340-12

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EXL DNA Polymerase

MATERIALS PROVIDED

Materials provided	Quantity		
	Catalog #600340 (100 U)	**Catalog #600342 (500 U)**	**Catalog #600344 (1000 U)**
EXL DNA polymerase (5 U/ µl)	20 µl	100 µl	200 µl
10× EXL reaction buffer	1 ml	2 × 1 ml	4 × 1 ml
Dimethyl Sulfoxide (DMSO)	1 ml	1 ml	1 ml
Stabilizing Solution	500 µl	500 µl	500 µl

STORAGE CONDITIONS

All Components: –20°C

ADDITIONAL MATERIALS REQUIRED

Thin-walled PCR tubes
DNase-, RNase-, and protease-free mineral oil (Sigma-Aldrich, Inc., Product Number M 5904)
Deoxynucleoside triphosphates (dNTP's)
DNase-/RNase-free, disposable micropipet tips

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; 6,444,428, and patents pending. 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 Agilent Technologies, 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

EXL DNA polymerase* provides superior performance in amplifying complex targets of lengths greater than 20 kb and provides higher fidelity than *Taq* DNA polymerase and *Taq*-based blends. EXL DNA polymerase features a special DNA polymerase composition that has been optimized specifically for extremely long targets. Our EXL DNA polymerase is provided with an optimized buffer, DMSO and a stabilizing solution to facilitate extreme reaction conditions needed for very long targets. EXL DNA polymerase provides robust yield, specificity, and reliable amplification of extra long targets. As a result, the EXL DNA polymerase can be used to successfully amplify 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 EXL 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.[1] Once incorporated, dU-containing DNA inhibits *Pfu* and most archaeal proofreading DNA polymerases, such as Vent® and Deep Vent® DNA polymerases, limiting their efficiency.[1] The ArchaeMaxx factor functions as a dUTPase, converting poisonous dUTP to harmless dUMP and inorganic pyrophosphate, resulting in improved overall PCR performance.

- 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.

CRITICAL OPTIMIZATION PARAMETERS

All PCR amplification reactions require optimization to achieve the highest product yield and specificity. Critical optimization parameters for successful PCR using the EXL DNA polymerase for amplification of targets >20 kb are outlined in the Table I and discussed in the following section. These include the quality and concentration of DNA template, DMSO usage, the appropriate concentration of dNTPs, sufficient enzyme concentration, and optimal cycling parameters.

TABLE I

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

Parameter	Genomic Targets (High-Complexity DNA)	Vector Targets (Low-Complexity DNA)
Input template	250–1000 ng genomic DNA	15–60 ng vector DNA
EXL DNA polymerase	5.0 U	5.0 U
DMSO concentration[a]	0–3% for targets <23 kb 3–6% for targets >23 kb	5–7% for targets >30 kb
dNTP concentration	500 µM each dNTP (2 mM total)	500 µM each dNTP (2 mM total)
Primers (each)	~200 ng (0.5 µM)	~200 ng (0.5 µM)
Extension time	1 min per kb	≥1 min per kb
Extension temperature	68°C	68°C
Denaturing temperature	92°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

Template Quality

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. 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.

Template Concentration

For amplifying genomic DNA templates, we recommend using 250 ng of template. 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 15–60 ng.

Enzyme Concentration

Robust product yield requires an adequate DNA polymerase concentration. Longer templates require 5 U of the EXL 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. The degree to which DMSO enhances product yield and specificity varies according to target length and complexity, so the DMSO concentration must be titrated for each template. For genomic DNA, 0–3% DMSO for targets ≤23 kb and 3–6% DMSO for targets >23 kb is generally recommended. For lambda DNA, 5–7% DMSO for targets >30 kb is generally suggested. The DMSO concentration should be titrated in the specified range in 1% increments.

Deoxynucleotide Concentrations

Amplification efficiencies are influenced by deoxynucleotide (dNTP) concentrations. Insufficient concentrations of dNTPs may result in lower yields. the use of 500 µM each dNTP is optimal.

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 ~0.5 µM final concentration of each primer. When using 25-mer oligonucleotide primers in a 50-µl reaction volume, this is equivalent to ~200 ng of each primer.

Cycling Parameters

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

Extension Time

We suggest maintaining 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 68°C should be used.

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.

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 a target ≥20 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
Distilled water	X µl to final volume of 50.0 µl
10×EXL reaction buffer	5.0 µl
dNTP mix (25 mM of each dNTP)	1.0 µl
DNA template: Genomic DNA Low-complexity templates (λ DNA or cloned DNA)	 250–1000 ng 15–60 ng
Primer #1	200 ng
Primer #2	200 ng
EXL DNA polymerase (5 U/µl)	1.0 µl
DMSO[a]	Either 0–3%, 3–6%, or 5–7%
Stabilizing solution[b]	1 µl
Total reaction volume	50.0 µl

[a] The optimal DMSO concentration must be determined by titration in 1% increments for each primer-template set.

[b] For a 50-µl reaction volume, 1 µl of stabilizing solution is optimal. Best results are obtained when the recommended volume of stabilizing solution is used.

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

3. Overlay each reaction with ~50 µl of DNase-, RNase-, and protease-free 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 and (B) Robocycler temperature cyclers.

(A)

Single-Block Temperature Cyclers

Segment	Number of cycles	Temperature	Duration
1	1	92°C	2 minutes
2	10	92°C	10 seconds
		Primer T_m – 5°C[a]	30 seconds
		68°C	60 seconds/kb of PCR target
3	20	92°C	10 seconds
		Primer T_m – 5°C	30 seconds
		68°C	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)

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	60 seconds/kb of PCR target
3	1	68°C	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.

5. Analyze the PCR amplification products by electrophoresis using an appropriate percentage acrylamide or agarose gel. Long PCR products greater than 20 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 EXL DNA 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, high molecular weight, 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 targets ≥ 20 kb, denaturation times of 10 seconds for single-block temperature cyclers or 30 seconds for RoboCycler 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
	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
	Adjust the ratio of primer versus template to optimize yield of the desired product
	Use thin-wall PCR tubes for Stratagene 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
	Use DMSO in the PCR mixture; titrate the DMSO concentration in 1% increments
Artifactual PCR smears	Decrease the amount of EXL DNA polymerase
	Optimize the cycling parameters specifically for the primer–template set and the thermal cycler used
	Reduce the extension time
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

REFERENCE

1. 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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EXL DNA Polymerase

Catalog #600340, 600342, 600344

QUICK-REFERENCE PROTOCOL

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

Component	Quantity per reaction
Distilled water	X µl to final volume of 50.0 µl
10×EXL reaction buffer	5.0 µl
dNTP mix (25 mM of each dNTP)	1.0 µl
DNA template: Genomic DNA Low-complexity templates (λ DNA or cloned DNA)	 250–1000 ng 15–60 ng
Primer #1	200 ng
Primer #2	200 ng
EXL DNA polymerase (5 U/µl)	1.0 µl
DMSO[a]	Either 0–3%, 3–6%, or 5–7%
Stabilizing solution[b]	1 µl
Total reaction volume	50.0 µl

[a] The optimal DMSO concentration must be determined by titration in 1% increments for each primer-template set.

[b] For a 50-µl reaction volume, 1 µl of stabilizing solution is optimal. Best results are obtained when the recommended volume of stabilizing solution is used.

- 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
  (B) RoboCycler temperature cyclers

(A)

Single-Block Temperature Cyclers

Segment	Number of cycles	Temperature	Duration
1	1	92°C	2 minutes
2	10	92°C	10 seconds
		Primer T_m – 5°C[a]	30 seconds
		68°C	60 seconds/kb of PCR target
3	20	92°C	10 seconds
		Primer T_m – 5°C	30 seconds
		68°C	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)

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	60 seconds/kb of PCR target
3	1	68°C	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.

- Analyze the PCR amplification products by gel electrophoresis