

# PyroPhage<sup>®</sup> 3173 DNA Polymerase, Exonuclease Minus (Exo<sup>-</sup>)

**IMPORTANT!**  
**-20°C Storage Required**  
**Immediately Upon Receipt**

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## Technical Support

Lucigen is dedicated to the success and satisfaction of our customers. Our products are tested to assure they perform as specified when used according to our recommendations. It is imperative that the reagents supplied by the user, especially the DNA targets to be cloned, are of the highest quality. Please follow the manual carefully and contact our technical service representatives if additional information is necessary. We encourage you to contact us with your comments regarding the performance of our products in your applications. Thank you.

## Product Designations

### PyroPhage 3173 DNA Polymerase, Exonuclease Minus (Exo<sup>-</sup>)

Product	Size	Catalog number
PyroPhage 3173 DNA Polymerase, Exo <sup>-</sup> (5U/μl)	500 U	30053-1
	5 x 500 U	30053-2
	10 x 500 U	30053-3

## Components and Storage

### Store at -20°C

Description	500 U	2500 U	5000 U
PyroPhage 3173 DNA Polymerase, Exo <sup>-</sup>	100 μl	5 x 100 μl	10 x 100 μl
PyroPhage 3173 2X PCR Buffer	2 x 1.25 ml	10 x 1.25 ml	20 x 1.25 ml
PCR Control pUC19 template plus primers (5 ng/μl template, 25 pmol/μl each primer)	5 μl	5 x 5 μl	10 x 5 μl

### Related products available separately

Product	Size	Catalog number
PyroPhage 3173 DNA Polymerase, Wild Type (5U/μl)	500 U	30051-1
	2500 U	30051-2
	5000 U	30051-3
PyroPhage 3173 2X PCR Buffer, 500 rxns	12.5 ml (10 x 1.25 ml)	30072-1
2.5 mM dNTP Mix, PCR Grade	2 ml (2 X 1ml)	30030-1
	10 ml (10 X 1ml)	30030-2
	20 ml (2 X 1ml)	30030-3
10 mM dNTP Set, PCR Grade	750 μl of each dNTP	30029-1
	5 X 750 μl of each dNTP	30029-2

## Product Description

PyroPhage 3173 DNA Polymerase, Exonuclease Minus (Exo<sup>-</sup>) is a thermostable bacteriophage enzyme that has an efficient reverse transcription activity. The thermostability of PyroPhage 3173 DNA Polymerase, Exo<sup>-</sup> makes it robust for thermocycling, enabling single-tube, single-enzyme reverse transcription PCR of RNA templates.

PyroPhage 3173 DNA Polymerase, Exo<sup>-</sup> is capable of PCR amplification of fragments greater than 1 kb. However, we have found that RT-PCR amplification of fragments greater than 400 bp in length is poor and is not recommended.

The PyroPhage 3173 DNA Polymerase has an inherent 3' - 5' exonuclease (proofreading) activity that has been inactivated in the PyroPhage 3173 DNA Polymerase, Exo<sup>-</sup> mutant resulting in a non-proofreading enzyme with a fidelity similar to that of Taq polymerase. The products amplified by PyroPhage 3173 DNA Polymerase, Exo<sup>-</sup> contain a single 3'-A or G overhang, like PCR products amplified by Taq polymerase, allowing direct cloning into any vector with complementary 3' overhangs.

As with most PCRs, amplification of particular primer/template sets can result in spurious products, particularly mispriming artifacts. Such variability is significantly reduced by use of alternative primer sets and optimized cycling conditions including cold reaction set-up. See Amplification Guidelines for PyroPhage 3173 DNA Polymerase Exo<sup>-</sup>.

## PyroPhage<sup>®</sup> 3173 DNA Polymerase, Exo<sup>-</sup>

The PyroPhage 3173 2X PCR Buffer contains a thermoprotectant for PCR thermocycling applications. In all cases, deoxyribonucleotide triphosphates (dNTPs) must be supplied separately.

## PyroPhage 3173 DNA Polymerase, Exo<sup>-</sup> for PCR

PyroPhage 3173, Exo<sup>-</sup> DNA Polymerase PCR routinely amplifies templates > 1 kb.

### PCR Reaction - Assemble on ice

25.0 µl	PyroPhage 3173 2X PCR Buffer
4.0 µl	dNTPs (2.5 mM each, 200 nM final)
0.5 µl	Primer 1 (100 µM, 1 µM final)*
0.5 µl	Primer 2 (100 µM, 1 µM final)*
X µl	Template DNA (0.01 ng to 100 ng)
0.5 µl	PyroPhage 3173 DNA Polymerase, Exo <sup>-</sup>
Y µl	H <sub>2</sub> O
50.0 µl	Total volume

## PyroPhage 3173 DNA Polymerase, Exo<sup>-</sup> for Reverse Transcription PCR (RT-PCR)

PyroPhage 3173 DNA Polymerase, Exo<sup>-</sup> is capable of both reverse transcription and subsequent PCR in a single tube.

### Reverse Transcription PCR Reaction - Assemble on ice

0.01 to 100 ng	Total RNA
25.0 µl	PyroPhage 3173 2X PCR Buffer
4.0 µl	dNTPs (2.5 mM each, 200 nM final)
1.0 µl	Primer 1 (10 µM, 200 nM final)*
1.0 µl	Primer 2 (10 µM, 200 nM final)*
0.5 µl	PyroPhage 3173 DNA Polymerase, Exo <sup>-</sup>
X µl	Nuclease-free H <sub>2</sub> O
50.0 µl	Total volume

### Cycling Conditions:

Step	Three Step Cycling		Two Step Cycling		# of Cycles
	Temp.	Time	Temp.	Time	
Initial Denature	94°C	2 mins	94°C	2 mins	1
Denature	94°C	15 secs	94°C	15 secs	25-40
Anneal*	50-72°C	15 secs	72°C	1 min/kb	
Extend	72°C	1 min/kb			
Final Extension	72°C	5-10 mins	94°C	5-10 mins	1
Hold	4°C	Indefinitely	4°C	Indefinitely	

\*See Amplification Guidelines, F.

## Amplification Guidelines for PyroPhage 3173 DNA Polymerase, Exo-

### A. Cold Reaction Set-Up

All reactions using PyroPhage 3173 DNA Polymerase, Exo- must be set up on ice and maintained at 4°C prior to amplification. Add PyroPhage 3173 DNA Polymerase, Exo- last to reactions just prior to incubation/cycling. The PyroPhage 3173 DNA Polymerase, Exo- has residual activity at temperatures above 4°C that can cause non-specific background amplification.

### B. Denaturation

A 2 minute initial denaturation step at 94°C is recommended although as little as 15 seconds may be sufficient. Subsequent denaturation steps should be 5 to 15 seconds.

### C. Annealing

Optimize the reaction conditions by performing the reaction starting with an annealing temperature 5°C below the calculated melting temperature of the primers. Vary the annealing temperature to optimize for the primer/template combination. Reducing the annealing temperature improves yield, while increasing annealing temperature improves specificity. The annealing step is typically 15 seconds. Use of two-step PCR cycling can improve specificity especially with RT-PCR.

### D. Extension

The optimal extension reaction is performed at 72°C. Allow 1 minute for every 1 kb to be amplified. A final extension of 5 to 10 minutes at 72°C may increase PCR product yield.

### E. Cycle Number

Between 25 to 30 cycles of PCR results in optimal amplification of desired products from more concentrated templates. Up to 40 cycles may be performed for detection of low-copy targets.

### F. Primers

#### Guidelines for primer selection:

The recommended PCR primer length is 15 to 40 nucleotides. Longer primers with higher annealing temperatures provide higher specificity and allow for two-step cycling.

Typical PCR primer design guidelines should be followed. Preferred GC content is 40-60%, distributed uniformly throughout the primer. Avoid the use of multiple G or C nucleotides at the 3'-end of the primer, as these may promote nonspecific priming. The primer should be free of inverted repeats, as these promote the formation of hairpins. The 3' end of the primer should also not be complementary to itself or to any other primer in the reaction mixture, to avoid primer self-annealing. The melting temperature of the primers should not differ by more than 5°C. If degenerate primers are used, at least 3 conservative nucleotides must be located at the 3' end of the primer. The Primer3 program (available at: <http://frodo.wi.mit.edu/>) has been successfully used to design primers for use with the PyroPhage 3173 DNA Polymerase, Exo-.

#### Estimation of the melting and annealing temperatures of primer:

If the primer is shorter than 25 nucleotides, the melting temperature ( $T_m$ ) can be approximated using the following formula:  $T_m$  (°C) = 4 (G + C) + 2 (A + T), where G, C, A, T = number of specified nucleotides in the primer. Initial annealing temperature should be 5°C lower than the melting temperature. More accurate melting temperature predictions may be obtained using specialized computer programs or web-based tools (e.g. Primer3, above) where the interactions of adjacent bases, the influence of salt concentration, etc. can be evaluated.

#### Primer Concentration

Primer concentrations from 200 nM to 1 μM are recommended for PCR. A primer concentration of 200 to 500 nM is recommended for RT-PCR.

### G. Template

Concentrations of 0.01 to 10 ng of plasmid or phage DNA or 10 to 100 ng of genomic DNA is recommended in a typical reaction volume of 50 μl. Total RNA template should be used at concentrations from 5 fg to 100 ng depending on the abundance of the target RNA.

If fidelity is crucial, higher initial template concentrations with a limited number of PCR cycles are recommended. Extremely high concentrations of template can inhibit both RT and PCR activity and may lead to generation of undesired products.

## PyroPhage<sup>®</sup> 3173 DNA Polymerase, Exo<sup>-</sup>

Most routine methods of template DNA or RNA purification are sufficient (e.g. phenol/chloroform or guanidine/silica-based methods). However, trace amounts of DNA purification agents (phenol, EDTA, Proteinase K, ethanol, etc.) may inhibit PCR. It is recommended that the template be dissolved in water or EDTA-free buffer rather than TE following purification.

### H. Reaction Overlay

A thermal cycler with a heated lid should be used to prevent evaporation of the PCR reaction mix. If no such lid is available, the reaction mixture can be overlaid with one-half reaction volume of PCR-grade mineral oil or paraffin (melting temperature 50-60°C).

### I. Magnesium

Magnesium is contained in the PyroPhage 3173 PCR Buffer at 2.0 mM final 1X concentration. If the DNA samples contain EDTA or other chelators, additional magnesium sulfate can be added. It is best to avoid the use of EDTA by dissolving the template in water rather than TE. Some primers may exhibit better sensitivity and performance with addition of 1 to 2 mM additional magnesium sulfate.

### J. dNTPs

Required dNTPs are NOT included in the buffer provided with the kit and must be added to a final concentration of 0.1 to 0.2 mM each. PCR Grade dNTPs are available from Lucigen, please see page 4. Addition of more dNTP beyond 0.2 mM will chelate available magnesium in the supplied buffer. Therefore additional magnesium sulfate should be supplemented when dNTPs are used at concentrations above 0.2 mM each.

## Appendices

### Appendix A: Biochemical characteristics of PyroPhage 3173 DNA Polymerase, Exo-

3'-5' exonuclease	none
5'-3' exonuclease	none
Thermostability (T <sub>1/2</sub> @95°C in 1X PCR buffer)	10 min.
K <sub>m</sub> dNTPs	40 μM
K <sub>m</sub> DNA	5.3 nM
Processivity	47 nt
3' Ends of Amplicons	single base A and G overhangs

# PyroPhage<sup>®</sup> 3173 DNA Polymerase, Exo<sup>-</sup>

## Appendix B. PyroPhage Buffer and Quality Control Assays

**PyroPhage 3173 2X PCR Buffer:** 40 mM Tris-HCl, 20 mM (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 20 mM KCl, 4 mM MgSO<sub>4</sub>, 0.2% Triton X-100, thermoprotectant, pH 8.8 @ 25°C.

### Activity Assay

Polymerase activity is assayed at 70°C with 0.2 mM each of dATP, dGTP, dTTP, dCTP (mix of unlabeled and [<sup>33</sup>P] dCTP); 10 µg activated calf thymus DNA, and 0.1 mg/ml BSA in a final volume of 50 µl.

### Absence of Endonuclease

PyroPhage 3173 DNA Polymerase, Exo<sup>-</sup> is determined to be free of detectable endonuclease or nicking activity. Supercoiled plasmid DNA is incubated with enzyme for 16 hours at 70°C. Agarose gel electrophoresis shows no alteration in mobility consistent with endonuclease or nicking activity.

### Absence of Exonuclease

PyroPhage 3173 DNA Polymerase Exo<sup>-</sup> is tested to be free of contaminating exonuclease activity by incubating Hind III-digested lambda DNA with enzyme at 70°C for 16 hours. Agarose gel electrophoresis shows no alteration in mobility consistent with exonuclease activity.

### Absence of Ribonuclease

PyroPhage 3173 DNA Polymerase, Exo<sup>-</sup> is tested to be free of contaminating RNase activity by incubating with a fluorogenic RNase substrate for 1 hour at 37°C. No increase in assay fluorescence above background was detected.

### Functional Assay

PyroPhage 3173 DNA Polymerase, Exo<sup>-</sup> is tested for performance in PCR using the PyroPhage 3173 PCR Buffer to amplify a 2 kb region of the *endA* gene from 2 X 10<sup>6</sup> molecules (10 ng) of *E. coli* gDNA. The resulting PCR product is visualized on an ethidium bromide-stained agarose gel.

## Appendix C. Control PCR

A control primer/template mix is supplied to allow functional testing of PyroPhage 3173 DNA Polymerase, Exo<sup>-</sup> in PCR. Amplification using this primer/template set results in an 860-bp amplicon of the pUC19 β-lactamase (*amp<sup>R</sup>*) gene.

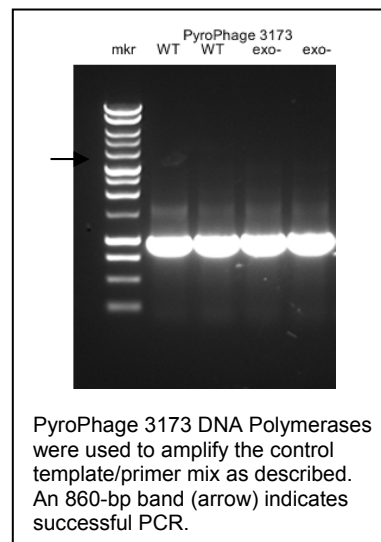
### Control PCR Reaction

25 µl	PyroPhage 3173 2X PCR Buffer
4 µl	dNTPs (2.5 mM each)
5 µl	Template DNA plus primers
1 µl	PyroPhage 3173 DNA Polymerase, Exo <sup>-</sup>
15 µl	Nuclease-free H <sub>2</sub> O
50 µl	Total volume

### Cycling Conditions:

Pre-heat thermal cycler to 94°C.

Denature	2 min. at 94°C.	X 1 cycle
Denature	15 sec. at 94°C	X 25 cycles
Anneal	15 sec. at 60°C	
Extend	1 min at 72°C	
Final Extension	10 min. at 72°C	X 1 cycle
Hold	Indefinitely at 4°C	



Analyze 5 µl of the reaction by agarose gel electrophoresis. A distinct band at 860 bp should be visible.

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