invitrogen™

Pfx50™ DNA Polymerase

Cat. no. 12355-012 **Size: 100 Reactions**
12355-036 **500 Reactions**

Conc. 5 U/µl **Store at -20°C**

Description

Pfx50™ DNA Polymerase is a fusion enzyme consisting of recombinant DNA polymerase from the archaean *Thermococcus zilligii* fused to an accessory protein. The highly thermostable polymerase possesses a proofreading 3′→ 5′ exonuclease activity, while the accessory protein stabilizes primer-template complexes in PCR.

Pfx50™ DNA Polymerase offers 50 times better fidelity than *Taq* DNA polymerase, coupled with high specificity and an extremely fast elongation rate (as fast as 15 seconds per kb). In addition, the fusion enzyme has an intrinsic hot-start capability for room-temperature reaction assembly.

10X *Pfx50*™ PCR buffer contains $MgSO_4$ at a final 1X concentration of 1.2 mM. A tube of 50-mM $MgSO_4$ is provided for further optimization. Reagents are provided for 100, 250 or 500 PCRs of 50 µl each.

Note:

- 10X *Pfx50*™ PCR buffer contains BSA; store at –20ºC.
- *Pfx50*™ DNA Polymerase produces blunt-end PCR products, which can be used with Directional TOPO® Cloning and Zero Blunt® TOPO® Cloning technologies.

Component	100 Rxn Kit	500 Rxn Kit
Pfx50™ DNA Polymerase (5 U/µl)	100 µl	500 µl
10X *Pfx50*™ PCR Mix	1.3 ml	2 × 1.3 ml
50-mM Magnesium Sulfate	1 ml	1 ml

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Pfx50™ DNA Polymerase Storage Buffer

20 mM Tris-HCl (pH 8.0), 40 mM KCl, 0.1 mM EDTA, 1 mM DTT, stabilizers, and 50% (v/v) glycerol

Unit Definition

One unit of *Pfx50*™ DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-insoluble material in 30 min at 74°C.

General Recommendations and Guidelines for PCR

General PCR parameters and troubleshooting information are documented in Innis, et al (Innis et al., 1990).

Template: *Pfx50*™ DNA Polymerase is suitable for amplifying targets up to 4 kb from the following templates:

Template	Amount
Genomic DNA	1–200 ng
Plasmid DNA	1–100 pg
cDNA	3–5 µl from 10 ng to 1 µg starting total RNA

Amplification of longer targets (up to 7 kb) may be possible, but may require more template and longer elongation times.

Primers: Use 0.3 µM per primer as a general starting point. For larger amounts of template (*e.g.*, 200 ng genomic DNA), increasing the concentration up to 0.5 µM per primer may improve yield.

Annealing Temperature: The annealing temperature is slightly higher than with typical PCR. **The optimal annealing temperature should be ~2°C lower than the T_m of the primers used. A range of 60–68°C is recommended.**

$MgSO_4$: $MgSO_4$ is included in the 10X *Pfx50*™ PCR Mix at a final 1X working concentration of 1.2 mM, which is sufficient for most templates. For further optimization, add 0.1 µl to 1.0 µl of 50-mM $MgSO_4$.

Extension Time: As little as 15 seconds per kb may be used; 30 seconds per kb is suitable for most targets. Use up to 60 seconds per kb for maximum yield.

PCR Protocol

The following procedure is suggested as a starting point when using *Pfx50*™ DNA Polymerase in any PCR amplification.

1. Program the thermal cycler as follows (see the note on annealing temperature on page 2):

   Initial denaturation: 94ºC for 2 minutes

   35 cycles of:
   Denaturation: 94ºC for 15 seconds
   Annealing: 60–68ºC (T_m of primers minus 2ºC) for 10–30 seconds
   Extension: 68ºC for 30–60 seconds per kb of PCR product

   Final extension: 68ºC for 5 minutes

2. Add the following components to an autoclaved microcentrifuge tube at room temperature (for multiple reactions, prepare a Master Mix of common components to enable accurate pipetting):

Component	Volume	Final Conc.
10X *Pfx50*™ PCR Mix	5 µl	1X
10 mM dNTP Mix	1.5 µl	0.3 mM each
Primer mix (10 µM each)	1.5 µl	0.3 µM each
Template DNA (see page 2 for amounts)	≥1 µl	As required
Pfx50™ DNA Polymerase (5 U/µl)	1 µl	5 units
Autoclaved, distilled water	to 50 µl	

3. Cap the tube, tap gently to mix, and centrifuge briefly to collect the contents.

4. Place the tube in the thermal cycler and run the program from Step 1. After cycling, maintain the reaction at 4ºC. Samples can be stored at –20ºC until use.

5. Analyze products using E-Gel® Pre-Cast agarose gels or standard agarose gel electrophoresis. Visualize by staining with SYBR Safe™ DNA gel stain or ethidium bromide.

Quality Control

Pfx50™ DNA Polymerase is tested in a PCR functional assay, a double-strand-endonuclease assay, and a 5´-exonuclease assay.

References

1. Innis, M. A., Gelfand, D. H., Sninsky, J. J., and White, T. S. (eds) (1990) *PCR Protocols: A Guide to Methods and Applications*, Academic Press, San Diego, CA

Additional Products

Product	Amount	Catalog no.
10 mM dNTP Mix, PCR Grade	100 µl	18427-013
10 mM dNTP Mix, PCR Grade	1 ml	18427-088
Invitrogen custom oligonucleotides	visit www.invitrogen.com	
E-Gel® 1.2% Starter Pak	6 gels plus PowerBase™	G6000-01
E-Gel® 1.2% 18-Pak	18 gels	G5018-01
TrackIt™ 1 Kb Plus DNA Ladder	100 applications	10488-085
TrackIt™ 100 bp DNA Ladder	100 applications	10488-058
SYBR Safe™ DNA gel stain (10,000X)	400 µl	S33102