

Tfi DNA Polymerase

Cat. No:

30342-011

30342-024

30342-052

Kit Size:

100 reactions

500 reactions

5000 reactions

Conc. 5 U/ μ l

Store at -20°C (non-frost-free)

Description

Tfi DNA Polymerase is purified from *E. coli* expressing cloned mutants of the *Thermus filiformis* DNA polymerase gene. This enzyme has both 5' \rightarrow 3' polymerase and 5' \rightarrow 3' exonuclease activity, but lacks 3' \rightarrow 5' exonuclease activity. *Tfi* DNA polymerase is heat-stable and will synthesize DNA at elevated temperatures from single-stranded templates in the presence of a primer (Shandilya et al., 2004).

Tfi DNA Polymerase can be used in protocols that currently use *Taq* DNA Polymerase without modification. PCR performance is comparable to that of *Taq* in yield, specificity, fidelity, and robustness. Like *Taq*, *Tfi* DNA Polymerase has a nontemplate-dependent terminal transferase activity that adds a single deoxyadenosine (A) to the 3' ends of PCR products.

Component	Kit Size		
	100 rxns	500 rxns	5,000 rxns
<i>Tfi</i> DNA Polymerase	100 μ l	500 μ l	5000 μ l
5X <i>Tfi</i> PCR Reaction Buffer	1.25 ml	4 \times 1.25 ml	50 ml
50 mM Magnesium Chloride	1 ml	1 ml	10 ml

Unit Definition

One unit of *Tfi* DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C.

Part no. 30342.pps

Rev. date: 6 Jul 2006

Guidelines for PCR

General PCR parameters and troubleshooting information are documented in Innis, et al (Innis et al., 1990). PCR reactions should be assembled in a DNA-free environment using clean, dedicated automatic pipettors and aerosol resistant barrier tips. Always keep the control DNA and other templates to be amplified isolated from the other components.

Basic PCR Protocol

The following protocol provides general guidelines for PCR amplification. Optimal reaction conditions—including incubation times and temperatures, and amounts of polymerase, primers, MgCl₂, and template DNA—may vary. Set up reactions on ice.

1. Program the thermal cycler as follows (note that the annealing temperature will vary depending on the T_m of your primers):

Initial denaturation: 94°C for 30 seconds–2 minutes

25–40 cycles of:

Denaturation: 94°C for 15–30 seconds

Annealing: T_m of primers minus 5°C for 30 seconds

Extension: 68–72°C for 1 minute per kb of PCR product

Final extension: 68–72°C for 10 minutes

2. Add the following components to a sterile 0.5-ml microcentrifuge tube **on ice**. For multiple reactions, prepare a master mix of common components to minimize reagent loss and enable accurate pipetting.

Component	Volume	Final Concentration
5X PCR Reaction Buffer	10 µl	1X
10 mM dNTP mix	1 µl	200 µM each
50 mM MgCl ₂	1.5 µl	1.5 mM
Primer mix (10 µM each)	1 µl	0.2 µM each
Template DNA	≥ 1 µl	as required
<i>Tfi</i> DNA Polymerase (5 U/µl)	1 µl	5 units
Autoclaved distilled water	to 50 µl	n/a

Protocol continued on next page

Basic PCR Protocol, continued

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, Basic PCR Protocol. After cycling, maintain the reaction at 4°C. Samples can be stored at -20°C until use.
5. Analyze the amplification products by agarose gel electrophoresis. We recommend using E-Gel® 1.2% gels and TrackIt™ 100 bp or 1kb Plus DNA ladders (see **Additional Products** on page 4).

“Hot-start” Protocol

In the “hot-start” method, *Tfi* DNA Polymerase is added after the initial denaturation step while the reaction temperature is held at 80°C, to ensure high specificity of the products being synthesized.

1. Program the thermal cycler as described in Step 1 of the Basic PCR Protocol, except after the initial denaturation step at 94°C, add a 2–5 minute hold step at 80°C (allow enough time to add polymerase to each tube).
2. Set up the reaction as described in Step 2 of the Basic PCR Protocol, except do *not* add *Tfi* DNA Polymerase to the reaction mix.
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. During the 80°C hold after initial denaturation, add 1 µl of *Tfi* DNA Polymerase to each reaction tube in the thermal cycler.
5. Continue with the thermal cycling program. After cycling, maintain the reaction at 4°C. Samples can be stored at -20°C until use.
6. Analyze the amplification products by agarose gel electrophoresis. We recommend using E-Gel® 1.2% gels and TrackIt™ 100 bp or 1kb Plus DNA ladders (see **Additional Products** on page 4).

Quality Control

Tfi DNA Polymerase is functionally tested for amplification and the absence of double- and single-stranded endonuclease activity, as well as the absence of contaminating exonuclease activity.

Additional Products

<u>Product</u>	<u>Amount</u>	<u>Catalog no.</u>
10 mM dNTP Mix, PCR Grade	100 µl	18427-013
10 mM dNTP Mix, PCR Grade	1 ml	18427-088
E-Gel [®] 1.2% Starter Pak	6 gels plus PowerBase [™]	G6000-01
E-Gel [®] 1.2% 18-Pak	18 gels	G5018-01
TrackIt [™] 100 bp DNA Ladder	100 applications	10488-058
TrackIt [™] 1kb Plus DNA Ladder	100 applications	10488-085

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
2. Shandilya, H., Griffiths, K., Flynn, E. K., Astatke, M., Shih, P. J., Lee, J. E., Gerard, G. F., Gibbs, M. D., and Bergquist, P. L. (2004) *Extremophiles* 8, 243-251

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