invitrogen

Platinum® *Tfi* Exo(-) DNA Polymerase

Cat. no: 60684-050 **Kit Size: 500 units**

Conc. 5 U/µl **Store at -20°C (non-frost-free)**

Description

Platinum® *Tfi* Exo(-) DNA Polymerase is recombinant *Tfi* Exo(-) DNA polymerase complexed with a proprietary antibody mix that inhibits polymerase activity at ambient temperature, allowing room-temperature reaction setup. Activity is restored after the initial denaturation step in PCR cycling at 94°C, providing an automatic "hot start" for increased specificity, sensitivity, and yield.

Tfi Exo(-) DNA Polymerase is purified from *E. coli.* expressing cloned mutants of the *Thermus filiformis* DNA polymerase gene. This enzyme has 5' → 3' polymerase activity, but lacks both 5' → 3' and 3' → 5' exonuclease activity for improved yield and robustness. *Tfi* Exo(-) 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).

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

Component	Amount
Platinum® *Tfi* Exo(-) DNA Polymerase	100 µl
5X Platinum® *Tfi* Reaction Buffer	4 × 1.3 ml
50 mM Magnesium Chloride	1 ml

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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.

General Recommendations for PCR Optimization

The protocol on the following page provides general guidelines for PCR amplification. Optimal reaction conditions—including incubation times and temperatures, and amounts of polymerase, primers, $MgCl_2$, and template DNA—may vary.

- For genomic DNA, 1.0 unit of Platinum® *Tfi* DNA Polymerase is sufficient for amplifying most targets less than 1kb. Increasing the amount of enzyme to 2.0 units may improve yield.
- For plasmid DNA, 1.0 unit is optimal.
- A general scheme for PCR optimization should start with adjusting the annealing temperature. The optimal annealing temperature should be 5–10° lower than the T_m of the primers used. For higher specificity, it may be necessary to gradually increase the annealing temperature in steps of 2–3°C.

Unit Definition

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

Basic PCR Protocol

Due to Platinum® *Tfi* Exo(-) DNA Polymerase's "hot-start" capability, the reaction can be set up at room temperature.

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

   Initial denaturation: 94ºC for 2 minutes

   25–40 cycles of:
   - Denaturation: 94ºC for 15–30 seconds
   - Annealing: T_m of primers minus 5–10ºC for 30 seconds
   - Extension: 68ºC for 1 minute per kb of PCR product

   Final extension: 68ºC for 10 minutes

2. Add the following components to a DNase/RNase-free microcentrifuge tube. For multiple reactions, prepare a master mix of common components to minimize reagent loss and enable accurate pipetting.

Component	Volume	Final Concentration
5X Platinum® *Tfi* Reaction Buffer	10 µl	1X
10 mM dNTP mix, PCR grade	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
Platinum® *Tfi* Exo(-) DNA Polymerase	0.2–0.4 µl	1–2 units*
Autoclaved distilled water	to 50 µl	n/a

   *May use up to 2 units for genomic DNA. See note on previous page.

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 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

Platinum® *Tfi* Exo(-) DNA Polymerase is evaluated in a DNA polymerization activity assay that measures the percent of polymerase inhibition versus an uninhibited control. Platinum® *Tfi* Exo(-) DNA Polymerase is also functionally tested for amplification and the absence of double- and single-stranded endonuclease activity, as well as the absence of exonuclease activity.

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
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