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Platinum® *Taq* DNA Polymerase High Fidelity

Cat. nos.	Size	Conc. 5 U/µL
11304-011	100 reactions	Store at –30°C to –10°C
11304-029	500 reactions	
11304-102	5,000 reactions	

Pub. Part no. 11304.pps MAN0000948 **Rev. Date** 18 Nov 2011

Description

Platinum® *Taq* DNA Polymerase High Fidelity is an enzyme mixture composed of recombinant *Taq* DNA polymerase, *Pyrococcus species* GB-D polymerase, and Platinum® *Taq* Antibody (Innis, 1988; Barnes, 1994). *Pyrococcus species* GB-D polymerase possesses a proofreading ability by virtue of its 3′ → 5′ exonuclease activity (Tindall, 1988). Mixing the proofreading enzyme with *Taq* DNA polymerase increases fidelity approximately six times more than that of *Taq* DNA polymerase alone, and it allows amplification of simple and complex DNA templates over a large range of target sizes. Targets 12–20 kb can be amplified with some optimization. Targets over 20 kb require additional optimization. The enzyme mixture is provided with an optimized buffer that improves enzyme fidelity and amplification of difficult templates.

The anti-*Taq* DNA polymerase antibody complexes with and inhibits polymerase activity at room temperature. Activity is restored after the initial denaturation step in PCR cycling at 94ºC, providing an automatic "hot start" for increased specificity, sensitivity, and yield (Chou, 1992; Sharkey, 1994).

Platinum® *Taq* DNA Polymerase High Fidelity is supplied at the same 5-unit per-µL concentration as Platinum® *Taq* DNA Polymerase. No modification to PCR reactions or protocols is necessary. Like regular *Taq*, Platinum® *Taq* DNA Polymerase High Fidelity has a nontemplate-dependent terminal transferase activity that adds a single deoxyadenosine (A) to the 3' ends of PCR products.

Product Use: For research use only.
Not intended for any animal or human therapeutic or diagnostic use.

Unit Definition

One unit of Taq DNA polymerase incorporates 10 nmol of deoxyribonucleotide into DNA in 30 minutes at 74°C.

Contents

Component	Kit Size		
	100 rxn	500 rxn	5,000 rxn
Platinum® Taq DNA Polymerase High Fidelity	20 µL	100 µL	1000 µL
10X High Fidelity PCR Buffer	1.25 mL	2.5 mL	50 mL
50 mM Magnesium Sulfate	1 mL	1 mL	25 mL

Platinum® *Taq* DNA Polymerase High Fidelity Storage Buffer

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

10X High Fidelity PCR Buffer

600 mM Tris-SO_4 (pH 8.9), 180 mM Ammonium Sulfate

Protocol

The following procedure is suggested as a guideline and starting point. The reaction size may be altered to suit user preference. Due to the "hot-start" capability of the polymerase, the reaction can be set up at room temperature.

1. Program the thermal cycler as follows:
   Initial denaturation: 94ºC for 30 seconds to 2 minutes
   25–35 cycles of:
   Denature: 94ºC for 15–30 seconds
   Anneal: 55°C for 15–30 seconds
   Extend: 68ºC for 1 minute per kb of PCR product

Note: Do not perform the initial denaturation for more than 30 seconds if the target is greater than 12 kb. We recommend an extension temperature of 68°C.

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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
10X High Fidelity PCR Buffer	5 µL	1X
10 mM dNTP mixture	1 µL	0.2 mM each
50 mM $MgSO_4$	2 µL	2 mM
Primer mix (10 µM each)	1–2 µL	0.2–0.4 µM each
Template DNA	≥1 µL	(as required)
Platinum® *Taq* High Fidelity	0.2 µL	1.0 unit*
Autoclaved, distilled water	to 50 µL	Not applicable

*1.0 unit is sufficient for amplifying most targets. In some cases, more enzyme may be required (up to 2.5 units). Use 1 unit of enzyme for targets above 12 kb

Note: For Genomic DNA and cDNA, use a primer concentration of 0.2 µM. For Plasmid and lambda DNA, increase the primer concentration to 0.4 µM

3. Cap the tube, tap gently to mix, and centrifuge it 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 –30ºC to –10°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).

References

Barnes, W.M. (1994) *Proc. Natl. Acad. Sci. USA* 91, 2216.

Chou, Q., Russel, M., Birch, D., Raymond, J., Bloch, W. (1992) *Nucl. Acids Res., 20*, 1717.

Innis, M.A., Myambo, K.B., Gelfand, D.H. and Brow, M.A.D. (1988) *Proc. Natl. Acad. Sci. USA 85*, 9436.

Sharkey, D.J., Scalice, E.R., Christy, K.G., Atwood, S.M., Daiss, J.L. (1994) *BioTechnology, 12*, 506.

Tindall, K.R. and Kunkel, T.A. (1988) *Biochemistry 27*, 6008.

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

Product Qualification and SDS

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/sds**.

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