Platinum® *Pfx* DNA Polymerase

Cat. No. 11708-013 **Size: 100 reactions**
11708-021 **250 reactions**
11708-039 **500 reactions**

Conc: 2.5 U/µl **Store at -20°C**

Note: Read and follow reaction conditions carefully to ensure optimal performance.

Description

Platinum® *Pfx* DNA Polymerase is a proprietary enzyme preparation containing recombinant DNA polymerase from *Thermococcus* sp. strain KOD (1,2). Platinum® *Pfx* DNA Polymerase possesses proofreading 3′ to 5′ exonuclease activity and provides higher fidelity than *Pfu* DNA polymerase (3). It is a highly processive enzyme with fast chain extension capability.

Platinum® *Pfx* DNA Polymerase is provided in inactive form, due to specific binding of the Platinum® antibody. Polymerase activity is restored after a PCR denaturation step at 94°C, providing an automatic "hot start" and increasing specificity, sensitivity, and yield (4). The high accuracy, specificity, and yield of Platinum® *Pfx* DNA Polymerase make it ideal for demanding PCR applications such as site-directed mutagenesis and PCR expression cloning.

For problematic and/or GC-rich templates, PCR_x Enhancer Solution is included with each kit (see the guidelines for use on page 2). The number of reactions per kit is based on a standard reaction size of 50 µl.

	Kit Size		
Components	**100 Rxns**	**250 Rxns**	**500 Rxns**
Platinum® *Pfx* DNA Polymerase	100 units	250 units	500 units
50 mM Magnesium Sulfate	1 ml	1 ml	1 ml
10X *Pfx* Amplification Buffer	1 ml	2 × 1 ml	3 × 1 ml
10X PCR_x Enhancer Solution	1 ml	2 × 1 ml	3 × 1 ml

Part no. 11708.pps Rev. date 07/11/03

This product is distributed for laboratory research only. CAUTION: Not for diagnostic use. The safety and efficacy of this product in diagnostic or other clinical uses has not been established.

For technical questions about this product, call the Invitrogen Tech-Line℠ U.S.A. 800 955 6288

Platinum® *Pfx* DNA Polymerase Storage Buffer

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

Unit Definition

One unit of Platinum® *Pfx* DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 min at 74°C.

Quality Control

The DNA polymerase:antibody complex is evaluated in a DNA polymerization activity assay that measures the percent of DNA polymerase inhibition versus an uninhibited control. Platinum® *Pfx* DNA Polymerase is functionally tested in an amplification using 100 ng of K562 genomic DNA as a template.

Guidelines for Using PCR_x Enhancer Solution

For problematic and/or GC-rich templates, PCR_x Enhancer Solution provides higher primer specificity, a broader range of optimal magnesium concentrations, broad annealing temperatures, and improved thermostability.

Use of PCR_x Enhancer Solution is optional; use in combination with 10X *Pfx* Amplification Buffer, not as a substitute. PCR_x Enhancer Solution lowers the DNA melting temperature (T_m), reducing the maximum primer annealing temperature approximately 2°C per 1X PCR_x Enhancer Solution concentration, while at the same time expanding the effective annealing temperature over a much broader range. To determine the optimal reaction concentrations and conditions, we recommend starting with an annealing temperature of 55°C to 60°C and varying the amount of 10X PCR_x Enhancer Solution. For targets with higher GC content (60 to 90%), we recommend testing 10X PCR_x Enhancer Solution at final concentrations of 0.5X, 1X, 2X, and 3X.

PCR Protocol

The following procedure is suggested as a guideline and starting point when using Platinum® *Pfx* DNA Polymerase in any PCR amplification.

1. Add the following components to an autoclaved microcentrifuge tube either at ambient temperature or on ice:

Component	Volume	Final Concentration
10X *Pfx* Amplification Buffer	5 µl	1X
10 mM dNTP mixture*	1.5 µl	0.3 mM each
50 mM $MgSO_4$	1 µl	1 mM
Primer mix (10 µM each)*	1.5 µl	0.3 µM each
Template DNA (10 pg - 200 ng)	≥1 µl	As required
Platinum® *Pfx* DNA Polymerase**	0.4–1 µl	1.0–2.5 units
Autoclaved, distilled water	to 50 µl	

   *Platinum® *Pfx* DNA Polymerase will not function in reactions that contain dUTP in either the dNTP mix or the primers.

   **For most targets 1 unit is sufficient. When amplifying targets above 3 kb, more enzyme may be required.

2. Mix tube contents and overlay with mineral or silicone oil, if necessary.
3. Cap the tube and centrifuge briefly to collect the contents.
4. Denature the template for 2 min at 94°C. Perform 25–35 cycles of PCR amplification as follows:

Three-step cycling	Two-step cycling
Denature: 94°C for 15 s	Denature: 94°C for 15 s
Anneal: 55°C for 30 s	Extend: 68°C for 1 min per kb
Extend: 68°C for 1 min per kb	

   Note: Two-step cycling can be used for long primers with high T_m.

5. Maintain the reaction at 4°C after cycling. Samples can be stored at -20°C until use.
6. Analyze the products by agarose gel electrophoresis.

References

1. Takagi, M., Nishioka, M., Kakihara, H., Kitabayashi, M., Inoue, H., Kawakami, B., Oka, M., and Imanaka, T. (1997) *Appli. Environ. Microbiol.*, *63*, 4504-4510.
2. Nishioka M, Mizuguchi H, Fujiwara S, Komatsubara S, Kitabayashi M, Uemura H, Takagi M, Imanaka T. (2001) *J. Biotechnol.*, *88,* 141–9.
3. Cline, J., Braman., and Hogrefe, H. H. (1996) *Nucleic Acid Res.*, *24*, 3546.
4. Sharkey, D.J., Scalice, E.R., Christy, K.G., Atwood, S.M., Daiss, J.L. (1994) *BioTechnology*, 12, 506.

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