

Platinum® *Pfx* DNA Polymerase

Cat. No. 11708-013
11708-021
11708-039

Size: 100 reactions
250 reactions
500 reactions

Conc: 2.5 U/μl

Store at -20°C

Description

Platinum® *Pfx* DNA Polymerase is a proprietary enzyme preparation containing recombinant DNA polymerase from *Thermococcus* species strain KOD (1,2). Platinum® *Pfx* DNA Polymerase possesses proofreading 3' to 5' exonuclease activity and is a highly processive enzyme with fast chain extension capability (3).

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” for increased specificity, sensitivity, and yield (4). Platinum® *Pfx* DNA Polymerase is 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.

Unit Definition

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

Kit Components

| | <u>100 Rxns</u> | <u>250 Rxns</u> | <u>500 Rxns</u> |
|--|-----------------|-----------------|-----------------|
| Platinum® <i>Pfx</i> DNA Polymerase | 100 units | 250 units | 500 units |
| 50 mM Magnesium Sulfate | 1 ml | 1 ml | 1 ml |
| 10X <i>Pfx</i> 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: 11 May 2010

Important Recommendations and Guidelines

- Platinum® *Pfx* DNA Polymerase will not work in reactions that contain dUTP in either the dNTP mix or the primers.
- Use a 1X final concentration of 10X *Pfx* Amplification Buffer as a general starting point. A higher final buffer concentration (1.5X–2X) may enhance reaction yield and specificity.
- We recommend using 1 unit of Platinum® *Pfx* for most targets. For targets above 2 kb, up to 1.25 units may be used. Note that more enzyme may be inhibitory.
- The recommended starting concentration of MgSO₄ is 1 mM.
- Use an annealing temperature of 55–60°C as a general starting point.
- Only use PCR_x Enhancer Solution for problematic and/or GC-rich templates.

Guidelines for Using PCR_x Enhancer Solution

PCR_x Enhancer Solution is included as an optional component for problematic and/or GC-rich templates. Use PCR_x Enhancer Solution 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 optimal concentrations and conditions, start with an annealing temperature of 55–60°C and varying the amount of 10X PCR_x Enhancer Solution. For targets with higher GC content (60–90%), test 10X PCR_x Enhancer Solution at final concentrations of 0.5X, 1X, 2X, and 3X.

Quality Control

The Certificate of Analysis (CofA) provides detailed quality control information for each product. CofAs are available on our website. Go to www.invitrogen.com/support and search for the CofA by product lot number, which is printed on the box.

PCR Protocol

The following protocol is specific for Platinum® *Pfx* DNA Polymerase. Follow these instructions carefully for optimal performance.

1. Add the following components to an sterile microcentrifuge tube at room temperature or on ice. The amounts are for a single reaction. Prepare a master mix of common components for multiple reactions.

| <u>Component</u> | <u>Volume</u> | <u>Final Concentration</u> |
|-------------------------------------|---------------|----------------------------|
| 10X <i>Pfx</i> Amplification Buffer | 5–10 µl * | 1X–2X |
| 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® <i>Pfx</i> DNA Polymerase | 0.4 µl | 1 unit |
| Autoclaved, distilled water | to 50 µl | |

*Use 5 µl (1X final concentration) of buffer as a general starting point.

2. Mix tube contents. Cap the tube and centrifuge briefly to collect the contents.
3. Denature the template for 2–5 minutes at 94°C. Note that a longer denaturation time (up to 5 minutes) may increase yield and specificity.
4. Perform 25–35 cycles of PCR amplification as follows:

Three-step cycling

Denature: 94°C for 15 seconds

Anneal: 55°C for 30 seconds

Extend: 68°C for 1 minute per kb

Two-step cycling

Denature: 94°C for 15 seconds

Extend: 68°C for 1 minute 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 *et. al.*, (1997) *Appl. Environ. Microbiol.*, 63, 4504-4510.
2. Nishioka *et. al.*, (2001) *J. Biotechnol.*, 88, 141-9.
3. Cline *et. al.*, (1996) *Nucleic Acid Res.*, 24, 3546.
4. Sharkey *et. al.*, (1994) *BioTechnology*, 12, 506.

Limited Use Label License No. 1: Thermostable polymerases

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,789,224, 5,618,711, and 6,127,155. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

Limited Use Label License No. 5: Invitrogen Technology

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. For products that are subject to multiple limited use label licenses, the terms of the most restrictive limited use label license shall control. Life Technologies Corporation will not assert a claim against the buyer of infringement of patents owned or controlled by Life Technologies Corporation which cover this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Life Technologies is willing to accept return of the product with a full refund. For information about purchasing a license to use this product or the technology embedded in it for any use other than for research use please contact Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008 ; Phone (760) 603-7200 or e-mail: outlicensing@lifetech.com.

Limited Use Label License No. 14: Direct Inhibition by Anti-polymerase Antibodies

Licensed to Invitrogen Corporation, under U.S. Patent Nos. 5,338,671; 5,587,287, and foreign equivalents for use in research only.

Limited Use Label License No. 136: Pfx DNA Polymerase

This product is manufactured for Invitrogen by Toyobo Co., Ltd.

©2010 Life Technologies Corporation. All rights reserved.

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