



PCRx Enhancer System

Cat. No. 11495-017

Size: 250 Reactions

Store at -20°C

Description:

The PCRx Enhancer System is an optimized buffer and cosolvent system that simplifies Polymerase Chain Reaction (PCR) amplification of problematic and/or GC-rich templates using standard dNTPs and thermocycling protocols. PCR optimization is often a difficult and time consuming process requiring adjustment of incubation times and temperature, magnesium, primer, dNTP, *Taq* DNA polymerase and DNA template concentration, and potentially, the design of multiple primer sets (1). In many circumstances, use of an automatic hot start (2,3), such as that provided by Platinum® *Taq* DNA Polymerase, results in higher specificity, wider magnesium optima, and improved yield for problematic templates. However, DNA sequences containing stable secondary structure(s) are frequently refractory to conventional PCR optimization strategies as these sequences are resistant to denaturation and pose barriers to primer annealing or processon of DNA polymerase.

PCRx Enhancer Solution is a novel PCR cosolvent that facilitates efficient amplification of GC-rich sequences and remedies difficulties associated with PCR of problematic templates. For problematic and/or GC-rich templates, the PCRx Enhancer System offers higher primer specificity, broader magnesium concentration optima, broader annealing temperature optima and improved thermostabilization of *Taq* DNA polymerase. The 10X PCRx Amplification Buffer and 10X PCRx Enhancer Solution are fully compatible with native and recombinant *Taq* DNA Polymerase (Cat. Nos. 18038-018 and 10342-020), Platinum® *Taq* DNA Polymerase (Cat. No. 10966-018), and Platinum® *Taq* DNA Polymerase High Fidelity (Cat. No. 11304-011). Enough reagents are supplied for 250 PCR amplifications of 50 µl each.

<u>Component</u>	<u>Part No.</u>	<u>Amount</u>
10X PCRx Enhancer Solution	52391	2 × 1 ml
50 mM MgSO ₄	52044	1 ml
10X PCRx Amplification Buffer	52395	2 × 1 ml

Doc. Rev: 012502

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.

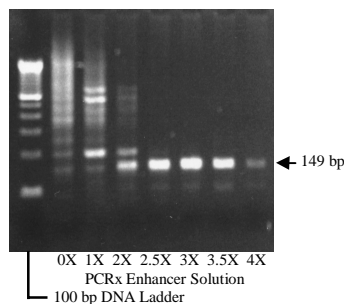
For technical questions about this product, call the Invitrogen Tech-LineSM U.S.A. 800 955 6288

Quality Control:

Each lot of the PCRx Enhancer System is evaluated in a functional PCR assay with Platinum® *Taq* DNA Polymerase and must demonstrate amplification of a 149-bp, (78.5% GC) trinucleotide repeat-containing sequence using 100 ng human genomic DNA as template.

General Recommendations and Guidelines:

- Conventional PCR buffer components in established protocols can be directly substituted with the 10X PCRx Amplification Buffer and 50 mM MgSO₄ resulting in more robust amplification and improved product yield in some cases. However, optimal reaction conditions vary and may need to be evaluated by the customer. Use of the PCRx Enhancer System results in wider reaction optima and significantly improves the probability of successful PCR amplification.



- 10X PCRx Enhancer Solution lowers DNA melting temperature (T_m).

Consequently, the maximum primer annealing temperature is lowered approximately 2°C per 1X PCRx Enhancer Solution concentration; however, effective annealing temperatures are widened over a much broader range. While no single thermal cycling protocol is optimal for every template, starting with an annealing temperature of 55°C to 60°C and varying the amount of 10X PCRx Enhancer Solution is recommended.

- Optimal concentration of 10X PCRx Enhancer Solution will vary depending on GC content, Mg⁺⁺ concentration and annealing temperature. For targets with 45 to 60% GC, testing of 10X PCRx Enhancer Solution at 0, 0.5X, and 1X final concentration is recommended. Targets with higher GC content (65 to 90%) may require up to 4X concentration.
- Addition of PCRx Enhancer Solution extends the range of effective Mg⁺⁺ concentration (1 to 3 mM). For most target sequences, best results are obtained using 1.5 mM MgSO₄. For targets with <45% GC content, use of 2 mM MgSO₄ is recommended.

NOTE: Primer sets that generate specific product using standard PCR buffer may not benefit from using PCRx Amplification Buffer.

- Best results with the PCRx Enhancer System have been obtained using 2.5 units of Platinum® *Taq* DNA Polymerase in a 50- μ l PCR mixture. For long templates (up to 10 kb) or six-fold higher fidelity, use of Platinum® *Taq* DNA Polymerase High Fidelity is recommended.

Basic PCR Optimization Protocol

The following basic protocol serves as a general guideline and a starting point. It is designed to test varying concentrations (0X to 4X) of PCRx Enhancer Solution. PCR reactions should be assembled in a DNA-free environment. Use of "clean" dedicated automatic pipettes and aerosol-resistant barrier tips are recommended. Always keep any DNA templates to be amplified isolated from the other components.

- For optimization, prepare enough master mix for seven reactions by adding the following components to an autoclaved microcentrifuge tube. Alternately, individual PCRx mixtures can be prepared according to the guidelines given below.

Components	Seven Rxn Master Mix	One Rxn	Final Concentration
10X PCRx Amplification Buffer	35 µl	5 µl	1X
10 mM dNTP Mixture	7 µl	1 µl	0.2 mM each
50 mM MgSO ₄	10.5 µl	1.5 µl	1.5 mM
Forward Primer (10 µM)	7 µl	1 µl	0.2 µM
Reverse Primer (10 µM)	7 µl	1 µl	0.2 µM
Template DNA	7 µl	1 µl	As required
Platinum [®] <i>Taq</i> DNA Polymerase (5 U/µl)	3.5 µl	0.5 µl	2.5 units/rxn
Autoclaved distilled water to	210 µl	30 µl	

- Add the following reagents to six thin-walled microcentrifuge tubes:

Component	Final PCRx Enhancer Solution Concentration					
	0	0.5X	1X	2X	3X	4X
Master mix	30	30	30	30	30	30
10X PCRx Enhancer Sol'n	-	2.5	5	10	15	20
Autoclaved distilled water	20	17.5	15	10	5	-

- Mix contents of tube and overlay (if required) with 50 µl of mineral or silicone oil.
- Incubate tubes in a thermal cycler at 95°C for 2 min to completely denature the template.
- Perform 25-35 cycles of PCR amplification as follows:
 - Denature 95°C for 30-45 s
 - Anneal 55°C-60°C for 30 s
 - Extend 68°C (1 min/kb)
- Analyze the amplification products by agarose gel electrophoresis. Visualize by ethidium bromide staining. Use appropriate molecular weight standards.

References:

1. Innis, M.A., Gelfand, D.H., Sninsky, J.J., and White, T.J., eds. (1990) *PCR Protocols: A Guide to Methods and Applications*, Academic Press, San Diego, CA.
2. Sharkey, D.J., Scalice, E.R., Christy, K.G., Atwood, S.M., Daiss, J.L. (1994) *BioTechnology*, 12, 506.
3. Westfall, B. *et al.* (1997) *Focus*[®] 19, 46.

Limited Label License No. 4:

This product is optimized for use in the Polymerase Chain Reaction (PCR) covered by patents owned by Roche Molecular Systems, Inc. and F. Hoffmann-La Roche, Ltd. ("Roche"). No license under these patents to use the PCR process is conveyed expressly or by implication to the purchaser by the purchase of this product. A license to use the PCR process for certain research and development activities accompanies the purchase of certain reagents from licensed suppliers such as Invitrogen, when used in conjunction with an Authorized Thermal Cycler, or is available from Applied Biosystems. Further information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404 or at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501.

Limited Label License No. 12:

The purchase of this product conveys to the buyer the non-transferable right to use the product and components of the product in research conducted by the buyer. The buyer cannot sell or otherwise transfer this product or its components to a third party and in particular, no rights are conveyed to the buyer to use the product or its components for commercial purposes. Commercial purposes means any activity for which a party receives 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 diagnostic purposes, or (4) resell the product or its components, whether or not such product or its components are resold for use in research. If purchaser is not willing to accept the limitations of this limited use statement, Invitrogen is willing to accept return of the products with a full refund. For information on purchasing a license to this product for purposes other than research, contact the Director of Licensing, 1600 Faraday Avenue, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500.