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 procession 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.

<table>
<thead>
<tr>
<th>Component</th>
<th>Part No.</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X PCRx Enhancer Solution</td>
<td>52391</td>
<td>2 x 1 ml</td>
</tr>
<tr>
<td>50 mM MgSO₄</td>
<td>52044</td>
<td>1 ml</td>
</tr>
<tr>
<td>10X PCRx Amplification Buffer</td>
<td>52395</td>
<td>2 x 1 ml</td>
</tr>
</tbody>
</table>

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.
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, Mg²⁺ 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 Mg²⁺ 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 µl PCR mixture. For long templates (up to 10 kb) or six-fold higher fidelity, use of Platinum® Taq DNA Polymerase High Fidelity is recommended.

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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.

1. 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.

<table>
<thead>
<tr>
<th>Components</th>
<th>Seven Rxn</th>
<th>Master Mix</th>
<th>One Rxn</th>
<th>Final Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>10X PCRx Amplification Buffer</td>
<td>35 µl</td>
<td>5 µl</td>
<td>1X</td>
<td></td>
</tr>
<tr>
<td>10 mM dNTP Mixture</td>
<td>7 µl</td>
<td>1 µl</td>
<td>0.2 mM each</td>
<td></td>
</tr>
<tr>
<td>50 mM MgSO₄</td>
<td>10.5 µl</td>
<td>1.5 µl</td>
<td>1.5 mM</td>
<td></td>
</tr>
<tr>
<td>Forward Primer (10 µM)</td>
<td>7 µl</td>
<td>1 µl</td>
<td>0.2 µM</td>
<td></td>
</tr>
<tr>
<td>Reverse Primer (10 µM)</td>
<td>7 µl</td>
<td>1 µl</td>
<td>0.2 µM</td>
<td></td>
</tr>
<tr>
<td>Template DNA</td>
<td>7 µl</td>
<td>1 µl</td>
<td>As required</td>
<td></td>
</tr>
<tr>
<td>Platinum® Taq DNA Polymerase (5 U/µl)</td>
<td>3.5 µl</td>
<td>0.5 µl</td>
<td>2.5 units/rxn</td>
<td></td>
</tr>
<tr>
<td>Autoclaved distilled water</td>
<td>210 µl</td>
<td>30 µl</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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

3. Mix contents of tube and overlay (if required) with 50 µl of mineral or silicone oil.
4. Incubate tubes in a thermal cycler at 95°C for 2 min to completely denature the template.
5. 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)
6. Analyze the amplification products by agarose gel electrophoresis. Visualize by ethidium bromide staining. Use appropriate molecular weight standards.
References:

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