invitrogen™

Platinum® PCR SuperMix High Fidelity

Cat. No. 12532-016 Size: 100 Reactions
Cat. No. 12532-024 Size: 5000 Reactions

Store at -20°C in a non-frost-free freezer.

Description:

Platinum® PCR SuperMix High Fidelity provides qualified reagents for high fidelity amplification of DNA templates by polymerase chain reaction (PCR). It is effective over a large range of target sizes—up to 15 kb genomic DNA. The mixture contains anti-*Taq* DNA polymerase antibody, Mg^{++}, deoxyribonucleotide triphosphates, recombinant *Taq* DNA polymerase, and *Pyrococcus* species *GB-D* thermostable polymerase. Anti-*Taq* DNA polymerase antibody inhibits polymerase activity, providing an automatic "hot start" (1,2) and permitting room temperature set-up (polymerase activity is restored after a denaturation step in PCR cycling at 94°C). Antibody-mediated hot starts improve PCR specificity and yield (3). *Pyrococcus* species *GB-D* polymerase possesses a proofreading ability by virtue of its 3′ to 5′ exonuclease activity (3). Mixture of the proofreading enzyme with *Taq* DNA polymerase increases fidelity approximately six times over that of *Taq* DNA polymerase alone.

Platinum® PCR SuperMix High Fidelity is supplied at 1.1X concentration to allow approximately 10% of the final reaction volume to be used for the addition of primer and template solutions. Reagents sufficient for 100 or 5000 amplification reactions of 50 µl each are provided.

Component:	100 Rxn Kit	5000 Rxn Kit
Platinum® PCR SuperMix High Fidelity*	4 × 1.125 ml	4 × 56.25 ml

*22 U/ml complexed recombinant *Taq* DNA polymerase, *Pyrococcus* species *GB-D* thermostable polymerase, and Platinum® *Taq* Antibody; 66 mM Tris-SO_4 (pH 8.9); 19.8 mM $(NH_4)_2SO_4$; 2.4 mM $MgSO_4$; 220 µM dNTPs; and stabilizers.

Part. No. 12532 Doc. Rev. 090502

Storage Conditions:

After thawing, Platinum® PCR SuperMix High Fidelity may be stored at 4°C for 3 months or -20°C for 1 year. Storage at 4°C avoids the necessity of thawing the mix before assembling the reaction. There is no detectable decrease in enzyme activity or performance after storage for 3 months at 4°C, or after 15 freeze-thaw cycles.

Recommendations and Guidelines:

- Because PCR is a powerful technique capable of amplifying trace amounts of DNA, take all appropriate precautions to avoid cross-contamination. Ideally, amplification reactions should be assembled in a DNA-free environment.
- Reactions may be assembled either at room temperature or on ice. We have observed no significant difference in reaction efficiency between these setup conditions.
- For multiple reactions, you can prepare a master mix of Platinum® PCR SuperMix High Fidelity and the component(s) common to all reactions.

PCR Protocol:

1. Add the following components in any order to each reaction tube:
   a. 45 µl Platinum® PCR SuperMix High Fidelity
   b. Primer solution (200 nM final concentration of each is recommended)*
   c. Template DNA solution (1–200 ng genomic DNA)*

   *A standard 50-µl PCR reaction includes a combined primer and template volume of 5 µl; we have observed no decrease in product yield if the amount of primer and template solution is between 1 µl and 15 µl.
2. Mix contents of tubes and cover with mineral or silicone oil if necessary.
3. Cap tubes and load in thermal cycler.
4. Incubate tubes at 94°C for 30 s to 2 min to completely denature the template and activate the enzyme.
5. Perform 25-35 cycles of PCR amplification as follows:

Denature	94°C for 15-30 s
Anneal	55°C for 15-30 s
Extend	68°C for 1 min per kb

Tips:

- If the PCR efficiency is not optimal, repeat the reaction with different primer concentrations from 100 to 500 nM, in 100 nM increments.
- For longer genomic DNA targets (>15 kb), we recommend adding 1–1.5 U of Platinum® *Taq* DNA Polymerase (Cat. no. 10966-018) to the reaction mix and increasing the extension time as specified (1 min per kb).
- At higher volumes of primer and template, the $MgSO_4$ concentration in the reaction will drop to suboptimal levels and yield will decrease. For combined primer-template volumes of >15 µl (in solution with 45 µl of Platinum® PCR SuperMix High Fidelity), we recommend adjusting the final $MgSO_4$ concentration in the reaction to 2.2 mM.

Quality Control:

Platinum® PCR SuperMix High Fidelity is evaluated in a DNA polymerization activity assay that measures the percent of *Taq* DNA polymerase inhibition versus an uninhibited control. A functional assay is also performed. Components are tested for the absence of DNase, RNase and exonuclease activities. Recombinant *Taq* DNA polymerase is tested for the absence of exonuclease, and double- and single-stranded endonuclease activities. The enzyme is >90% homogeneous as determined by SDS-polyacrylamide gel electrophoresis.

References:

1. Chou, Q., Russel, M., Birch, D., Raymond, J., Bloch, W. (1992) *Nucl. Acids Res.*, 20, 1717.
2. Sharkey, D.J., Scalice, E.R., Christy, K.G., Atwood, S.M., Daiss, J.L. (1994) *BioTechnology*, 12, 506.
3. Westfall, B., Sitaraman, K., Solus, J., Hughes, J., Rashtchian, A. (1997) *Focus®*, 19.2, 46.

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