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Platinum® PCR SuperMix

Cat. nos.	Size	Store at –30°C to –10°C
11306-016	100 reactions	
11306-081	5000 reactions	

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Description

Platinum® PCR SuperMix provides qualified reagents for the amplification of nucleic acid templates by polymerase chain reaction (PCR). The mixture contains anti-*Taq* DNA polymerase antibody, Mg^{++}, dNTPs, and recombinant *Taq* DNA polymerase at concentrations sufficient to allow amplification during PCR. Platinum® PCR SuperMix 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.

Anti-*Taq* DNA polymerase antibody inhibits polymerase activity providing an automatic "hot start" (Chou, 1992; Sharkey, 1994) and permits ambient temperature set-up. Antibody-mediated hot starts improve PCR specificity and yield (Westfall, 1997). Due to specific binding of the antibody, Platinum® PCR SuperMix is present in an inactive form and is reactivated after a denaturation step in PCR cycling at 94°C.

Platinum® PCR SuperMix may be stored at either –30°C to –10°C or 2°C to 8°C. Storage at 2°C to 8°C avoids the necessity of thawing the mix before assembling the PCR. No detectable reduction of PCR performance or enzyme activity is observed after storage of Platinum® PCR SuperMix for 12 months at 2°C to 8°C. Repeated freeze-thaw cycles do not reduce performance or activity.

Component	100-rxn size	5000-rxn size
Platinum® PCR SuperMix	4 × 1.125 mL	4 × 56.25 mL

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

Components

22 U/mL complexed recombinant *Taq* DNA polymerase with Platinum® *Taq* Antibody, 22 mM Tris-HCl (pH 8.4), 55 mM KCl, 1.65 mM $MgCl_2$, 220 μM dGTP, 220 μM dATP, 220 μM dTTP, 220 μM dCTP, and stabilizers.

Guidelines and Recommendations

Since PCR is a powerful technique capable of amplifying trace amounts of DNA, all appropriate precautions should be taken to avoid cross-contamination. Ideally, amplification reactions should be assembled in a DNA-free environment. Use of aerosol-resistant barrier tips is recommended. Take care to avoid contamination of PCR SuperMix with the primers or template DNA used in individual reactions. PCR products should be analyzed in an area separate from the reaction assembly area.

A standard 50-μL reaction uses 45 μL of Platinum® PCR SuperMix and 5 μL of primer and template solutions. For the primer sets used in the development of Platinum® PCR SuperMix, no decrease in product yield was observed if the amount of template and primer solution added is between a fraction of a microliter and 20 μL. Lower yield occurs as the Mg^{++} concentration drops to a suboptimal level. If the final Mg^{++} concentration is adjusted to 1.5 mM, the volume of primer and template solution that can be added to 45 μL of Platinum® PCR SuperMix can exceed 50 μL.

Protocol

The following protocol is suggested as a starting point and guideline when using Platinum® PCR SuperMix. We recommend assembling reactions on ice from pre-chilled components. This protocol is for a reaction size of approximately 50 µL. The reaction size may be adjusted as desired.

Note: For multiple reactions with common components, prepare a master mix of the components common to all reactions to reduce pipetting errors.

1. Set up the reaction tubes/plates on ice.
2. Add the following components in any order to each reaction vessel.
   - 45-µL Platinum® PCR SuperMix
   - Primers (200 nM final concentration per primer is recommended)*
   - Template DNA solution*

   *Total volume of primer and template solution can be 0.5–20 µL.
3. Cap reaction vessels and load in thermal cycler at 94°C.
4. Incubate tubes in a thermal cycler at 94°C for 30 seconds–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 seconds
Anneal:	55°C for 15–30 seconds
Extend:	72°C for 1 minute per kb

References

Chou, Q., Russel, M., Birch, D., Raymond, J., Bloch, W. (1992) *Nucl. Acids Res., 20*, 1717.

Sharkey, D.J., Scalice, E.R., Christy, K.G., Atwood, S.M., Daiss, J.L. (1994) *BioTechnology, 12*, 506.

Westfall, B., Sitaraman, K., Solus, J., Hughes, J., Rashtchian, A. (1997) *Focus®*, 19.2, 46.

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