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Platinum® *Taq* DNA Polymerase

Cat. No. 10966-050 **Size: 50 reactions**
Conc: 5 U/µl **Store at –20°C in a non-frost-free freezer**

Kit Contents

This sample kit includes sufficient reagents for 50 amplification reactions of 50 µl each. Ordering information for standard kit sizes is provided on page 4.

Component	Amount
Platinum® *Taq* DNA Polymerase (5 U/µl)	10 µl
10X PCR Buffer, Minus Mg	1.25 ml
50-mM Magnesium Chloride	1 ml

Description

Platinum® *Taq* DNA Polymerase is recombinant *Taq* DNA polymerase complexed with a proprietary antibody that blocks polymerase activity at ambient temperatures. Activity is restored after the denaturation step in PCR cycling at 94°C, thereby providing an automatic "hot start" for *Taq* DNA polymerase in PCR (1,2,3). Hot starts in PCR provide increased sensitivity, specificity, and yield, while allowing assembly of reactions at room temperature. The use of this antibody helps reduce PCR optimization requirements, reaction set-up time and effort, handling of reaction components, and contamination risk, thereby improving PCR results.

Platinum® *Taq* DNA Polymerase is supplied at the same 5 unit per µl concentration as *Taq* DNA Polymerase (Cat. No. 10342-053). No modification to PCR reactions or protocols are necessary. This enzyme formulation can also be used in larger volume cocktail mixes without difficulty.

Storage Buffer

20 mM Tris-HCl (pH 8.0), 40 mM NaCl, 2 mM Sodium Phosphate, 0.1 mM EDTA, 1 mM DTT, stabilizers, 50% (v/v) glycerol

Part No: 100004289 Rev. date: 26 Jun 2008

Quality Control

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

Unit Definition

One unit of Platinum® *Taq* DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 min at 74°C.

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.
- If the PCR efficiency is not optimal, repeat the reaction with different primer concentrations from 100 to 500 nM (final concentration), in 100-nM increments.
- A concentration of 1.5 mM $MgCl_2$ is sufficient for most targets. For further optimization, prepare a titration from 1.5 mM to 3 mM in 0.25-mM increments.
- For longer genomic DNA targets, we recommend using 2–2.5 U of Platinum® *Taq* DNA Polymerase and increasing the extension time as specified (1 min per kb).

Protocol

The procedure on the following page is suggested as a guideline and starting point when using Platinum® *Taq* DNA Polymerase in any PCR amplification. Optimal reaction conditions (incubation times and temperatures, concentration of Platinum® *Taq* DNA Polymerase, primers, $MgCl_2$, and template DNA) vary and need to be optimized. Reaction size may be altered to suit user preferences.

Protocol, continued

1. Add the following components to a sterile 0.5-ml microcentrifuge tube:

Components	Volume	Final Concentration
10X PCR Buffer, Minus Mg	5 µl	1X
10 mM dNTP mixture	1 µl	0.2 mM each
50 mM $MgCl_2$	1.5 µl	1.5 mM
Primer mix (10 µM each)	1 µl	0.2 µM each
Template DNA	≥1 µl	(as required)
Platinum® *Taq* DNA Polymerase	0.2 µl	1.0 unit*
Autoclaved, distilled water	to 50 µl	Not applicable

   *1.0 unit is sufficient for amplifying most targets. In some cases, more enzyme may be required (up to 2.5 units).

   If desired, a master mix can be prepared for multiple reactions, to minimize reagent loss and to enable accurate pipetting.

2. Mix contents of the tubes and overlay with 50 µl of mineral or silicone oil, if necessary.
3. Cap the tubes and centrifuge briefly to collect the contents.
4. Incubate tubes in a thermal cycler 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 30 s
Anneal	55°C for 30 s
Extend	72°C for 1 min per kb

6. Maintain the reaction at 4°C after cycling. The samples can be stored at -20°C until use.
7. Analyze the products by agarose gel electrophoresis and visualize by ethidium bromide staining. Use appropriate molecular weight standards.

Additional Products

The following products are available on our website at www.invitrogen.com or by contacting technical support.

	Cat. No.	Amount
Platinum® *Taq* DNA Polymerase	10966-018	100 Reactions
	10966-026	250 Reactions
	10966-034	500 Reactions
	10966-083	5,000 Reactions

References

1. Chou, Q., *et al*. (1992) *Nucl. Acids Res. 20*, 1717.
2. Sharkey, D.J., *et al*. (1994) *BioTechnology 12*, 506.
3. Westfall, B.A., *et al*. (1997) *Focus® 19.3*, 46.

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