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Platinum® *Taq* DNA Polymerase High Fidelity

Cat. No. 11304-050 **Size: 50 reactions**
Conc: 5 U/µl **Store at –20°C (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 High Fidelity (5 U/µl)	10 µl
10X High Fidelity PCR Buffer	1.25 ml
50-mM Magnesium Sulfate	1 ml

Description

Platinum® *Taq* DNA Polymerase High Fidelity is an enzyme mixture composed of recombinant *Taq* DNA polymerase, *Pyrococcus species* GB-D polymerase, and Platinum® *Taq* Antibody (1,2). *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 and allows amplification of simple and complex DNA templates over a large range of target sizes. Targets 12–20 kb can be amplified with some optimization. Targets over 20 kb require additional optimization. The enzyme mixture is provided with an optimized buffer that improves enzyme fidelity and amplification of difficult templates.

The anti-*Taq* DNA polymerase antibody complexes with and inhibits polymerase activity at room temperature. Activity is restored after the initial denaturation step in PCR cycling at 94ºC, providing an automatic "hot start" for increased specificity, sensitivity, and yield (4,5).

Platinum® *Taq* DNA Polymerase High Fidelity is supplied at the same 5 unit per µl concentration as Platinum® *Taq* DNA Polymerase. No modification to PCR reactions or protocols are necessary. Like regular *Taq*, Platinum® *Taq* DNA Polymerase High Fidelity has a nontemplate-dependent terminal transferase activity that adds a single deoxyadenosine (A) to the 3' ends of PCR products.

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Platinum® *Taq* DNA Polymerase High Fidelity Storage Buffer

20 mM Tris-HCl (pH 8.0), 0.1 mM EDTA, 1 mM DTT, stabilizers, and 50% (v/v) glycerol

10X High Fidelity PCR Buffer

600 mM Tris-SO_4 (pH 8.9), 180 mM Ammonium Sulfate

Unit Definition

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

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.

Notes

- Genomic DNA and cDNA: Use a primer concentration of 0.2 µM
- Plasmid and lambda DNA: Increase the primer concentration to 0.4 µM
- Do not perform the initial denaturation for more than 30 seconds if the target is greater than 12 kb.
- For longer genomic DNA targets (>15 kb), we recommend using 2–2.5 U of Platinum™ *Taq* DNA Polymerase High Fidelity and increasing the extension time as specified (1 min per kb).
- We recommend an extension temperature of 68°C
- A concentration of 2 mM $MgSO_4$ is sufficient for most targets. For further optimization, prepare a titration from 2 mM to 4 mM in 0.25-mM increments.

Protocol

The following procedure is suggested as a guideline and starting point. The reaction size may be altered to suit user preference. Due to the "hot-start" capability of the polymerase, the reaction can be set up at room temperature.

1. Program the thermal cycler as follows:

   Initial denaturation: 94ºC for 30 seconds to 2 minutes (see **Notes**, page 2)

   25–35 cycles of:
   - Denature: 94ºC for 15–30 seconds
   - Anneal: 55°C for 15–30 seconds
   - Extend: 68ºC for 1 minute per kb of PCR product

2. Add the following components to a DNase/RNase-free microcentrifuge tube. For multiple reactions, prepare a master mix of common components to minimize reagent loss and enable accurate pipetting.

Component	Volume	Final Concentration
10X High Fidelity PCR Buffer	5 µl	1X
10 mM dNTP mixture	1 µl	0.2 mM each
50 mM $MgSO_4$	2 µl	2 mM
Primer mix (10 µM each)	1–2 µl	0.2–0.4 µM each (see **Notes**)
Template DNA	≥1 µl	(as required)
Platinum® *Taq* High Fidelity	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).

3. Cap the tube, tap gently to mix, and centrifuge briefly to collect the contents.

4. Place the tube in the thermal cycler and run the program from Step 1. After cycling, maintain the reaction at 4ºC. Samples can be stored at –20ºC until use.

5. Analyze the amplification products by agarose gel electrophoresis. We recommend using E-Gel® 1.2% gels and TrackIt™ 100 bp or 1kb Plus DNA ladders (see **Additional Products** on page 4).

References

1. Innis, M.A., Myambo, K.B., Gelfand, D.H. and Brow, M.A.D. (1988) *Proc. Natl. Acad. Sci. USA 85*, 9436.
2. Barnes, W.M. (1994) *Proc. Natl. Acad. Sci. USA* 91, 2216.
3. Tindall, K.R. and Kunkel, T.A. (1988) *Biochemistry 27*, 6008.
4. Chou, Q., Russel, M., Birch, D., Raymond, J., Bloch, W. (1992) *Nucl. Acids Res., 20*, 1717.
5. Sharkey, D.J., Scalice, E.R., Christy, K.G., Atwood, S.M., Daiss, J.L. (1994) *BioTechnology, 12*, 506.

Additional Products

Product	Amount	Catalog no.
Platinum® *Taq* DNA Polymerase High Fidelity	100 rxns	11304-011
	500 rxns	11304-029
	5,000 rxns	11304-102
10 mM dNTP Mix, PCR Grade	100 µl	18427-013
10 mM dNTP Mix, PCR Grade	1 ml	18427-088
E-Gel® 1.2% Starter Pak	6 gels plus PowerBase™	G6000-01
E-Gel® 1.2% 18-Pak	18 gels	G5018-01
TrackIt™ 100 bp DNA Ladder	100 applications	10488-058
TrackIt™ 1kb Plus DNA Ladder	100 applications	10488-085

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