Invitrogen™
life technologies

Platinum® *Pfx* DNA Polymerase

Optimizing for greater yield – Questions and Answers

The purpose of this Technical Brief is to provide answers to some of the most commonly asked questions we are receiving on our Tech-Line℠ regarding Platinum® *Pfx* DNA Polymerase.

Sometimes I have seen ethidium bromide-stained DNA in the wells of my agarose gel following amplification with Platinum® *Pfx* DNA Polymerase. Why is this?

It is probably a combination of the following:

- Platinum® *Pfx* DNA Polymerase has more protein per unit of enzyme than other amplification enzymes.
- Platinum® *Pfx* DNA Polymerase may have different DNA-binding properties than other enzymes. If the protein remains bound to the DNA, migration is decreased in the gel.

If this reduces the yield of product migrating into the gel, add SDS to the sample loading buffer to a final concentration of 0.1%. Note that 10X BlueJuice™ Loading Buffer (Cat. No. 10816-015) does not contain SDS.

I substituted Platinum® *Pfx* DNA Polymerase for *PfuTurbo*® DNA Polymerase (or other proof-reading enzyme) in my current protocol, and I am not satisfied with the yield of Platinum® *Pfx* DNA Polymerase by comparison.

Platinum® *Pfx* DNA Polymerase may not perform to its full potential (e.g., priming specificity or yield) when the enzyme is "dropped" into a protocol optimized for another enzyme. Key differences include the use of more enzyme and the use of more magnesium than is optimal for Platinum® *Pfx* DNA Polymerase.

While you may use your *PfuTurbo*® DNA Polymerase conditions as a starting point, please note:

- Use the 10X *Pfx* Amplification Buffer since it is pre-optimized to work with Platinum® *Pfx* DNA Polymerase.
- You may need to lower your magnesium concentration (relative to other polymerases) to be in the range recommended for Platinum® *Pfx* DNA Polymerase.
- Use a lower annealing temperature than for *PfuTurbo*® DNA Polymerase since the magnesium concentration is lower for Platinum® *Pfx* DNA Polymerase.
- Use the recommended number of units of Platinum® *Pfx* DNA Polymerase (usually 1.25 units of *Pfx* is sufficient, compared to 2.5 units for *PfuTurbo*®).

I used the PCR_x Enhancer Solution as a substitute for the 10X *Pfx* Amplification Buffer and saw no signal when I looked at my PCR products on a gel.

PCR_x Enhancer Solution is not a substitute for the 10X *Pfx* Amplification Buffer, and must be used only in addition to the 10X buffer. It is recommended for use with GC-rich templates and/or nucleotide repeat sequences. If you are not amplifying GC-rich templates or nucleotide repeat sequences, the PCR_x Enhancer is not needed. Invitrogen recommends using PCR_x Enhancer Solution after the 10X *Pfx* Amplification Buffer has been tried.

See reverse side for additional information.

I compared Platinum® *Pfx* DNA Polymerase with an enzyme mix (a combination of *Taq* DNA Polymerase and a proofreading enzyme), and the Platinum® *Pfx* DNA Polymerase provided a lower yield than the enzyme mix. Why should I use Platinum® *Pfx* DNA Polymerase?

While enzyme mixes offer improved fidelity over *Taq* DNA Polymerase alone, Platinum® *Pfx* DNA Polymerase provides much higher fidelity since it is a proofreading polymerase (and not diluted by *Taq* DNA Polymerase). If yield is more important than fidelity, then an enzyme mix such as Platinum® *Taq* DNA Polymerase High Fidelity is a better choice of enzyme. Platinum® *Pfx* DNA Polymerase does give high yields relative to other proofreaders, but may not give as great a yield as enzyme mixes.

I am amplifying targets > 2 kb using Platinum® *Pfx* DNA Polymerase. Do you have any suggestions on how to optimize the reaction?

Here are suggestions for amplifying long sequences with Platinum® *Pfx* DNA Polymerase:

- Increase the extension time to 1 min/kb.
- Decrease the extension temperature to 68°C.
- Use 2.5 units (1 µl) of enzyme rather than the 1 unit suggested for shorter templates.
- If the primers being used for the amplification are long, i.e., > 45 bases, increase the magnesium concentration to 1.5 mM.

If you have encountered situations that are not represented here, please continue to contact Technical Services so we can address your needs. Your feedback will also be used in future technical briefs.

Ordering information

Description	Cat. No.	Size
Platinum® *Pfx* DNA Polymerase	11708-013	100 rxns
[Includes: 10X *Pfx* Amplification Buffer, 10X PCR_x Enhancer Solution, and 50 mM magnesium sulfate]	11708-021	250 rxns
	11708-039	500 rxns

[1, 2, 14, 37] Products mentioned above are subject to the Limited Label Licenses indicated by the superscript numbers. Please refer to the Invitrogen web site or catalog for Limited Use Label Licenses corresponding to the numbers indicated.

Corporate headquarters:
1600 Faraday Avenue • Carlsbad, CA 92008 USA • Tel: 760 603 7200 • Fax: 760 602 6500 • Toll Free Tel: 800 955 6288 • E-mail: tech_service@invitrogen.com • www.invitrogen.com
European headquarters:
Invitrogen Ltd, 3 Fountain Drive • Inchinnan Business Park • Paisley PA4 9RF, UK • Tel (Free Phone Orders): 0800 269 210 • Tel (General Inquiries): 0800 5345 5345
Fax: +44 (0) 141 814 6287 • E-mail: eurotech@invitrogen.com

711-021352 031802 xxM