

Platinum™ *Pfx* DNA Polymerase

Catalog Numbers 11708-013, 11708-021, and 11708-039

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WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](https://www.thermofisher.com/support).

Product description

The Invitrogen™ Platinum™ *Pfx* DNA Polymerase is a proprietary enzyme preparation containing recombinant DNA polymerase from *Thermococcus* species *KOD*. The Platinum™ *Pfx* DNA Polymerase possesses proofreading 3' to 5' exonuclease activity and is a highly processive enzyme with fast chain extension capability.

The Platinum™ *Pfx* DNA Polymerase is provided in inactive form, due to specific binding of the Platinum™ antibody. Polymerase activity is restored after a PCR denaturation step at 94°C, providing an automatic “hot start” for increased specificity, sensitivity, and yield. Platinum™ *Pfx* DNA Polymerase is ideal for demanding PCR applications such as site-directed mutagenesis and PCR expression cloning.

For problematic and/or GC-rich templates, PCR_x Enhancer Solution is included with each kit. See “Guidelines for PCR_x Enhancer Solution” on page 2.

Contents and storage

Contents	Cat. No.			Storage
	11708-013 (100 rxns)	11708-021 (250 rxns)	11708-039 (500 rxns)	
Platinum™ <i>Pfx</i> DNA Polymerase (2.5 U/μL)	100 U	250 U	500 U	-20°C
50 mM Magnesium sulfate	1 mL	1 mL	1 mL	
10X <i>Pfx</i> Amplification Buffer	1 mL	2 x 1 mL	3 x 1 mL	
10X PCR _x Enhancer Solution	1 mL	2 x 1 mL	3 x 1 mL	

For Research Use Only. Not for use in diagnostic procedures.

Note: Unit (U) definition: One unit of Platinum™ *Pfx* DNA Polymerase incorporates 10 nmol of deoxyribonucleotide into acid-precipitable material in 30 minutes at 74°C.

Procedural guidelines

- Platinum™ *Pfx* DNA Polymerase will not work in reactions that contain dUTP in the dNTP mix or the primers.
- Use a 1X final concentration of 10X *Pfx* Amplification Buffer as a general starting point. A higher final buffer concentration (1.5–2X) may enhance reaction yield and specificity.
- We recommend using 1 unit of Platinum™ *Pfx* for most targets. For targets above 2 kb, up to 1.25 units may be used. Note that more enzyme may be inhibitory.
- The recommended starting concentration of MgSO₄ is 1 mM.
- Use an annealing temperature of 55–60°C as a starting point.
- Only use PCR_x Enhancer Solution for problematic and/or GC-rich templates.

Guidelines for PCR_x Enhancer Solution

PCR_x Enhancer Solution is included as an optional component for problematic and/or GC-rich templates. Use PCR_x Enhancer Solution in combination with 10X *Pfx* Amplification Buffer, *not* as a substitute.

PCR_x Enhancer Solution lowers the DNA melting temperature (T_m), reducing the maximum primer annealing temperature approximately 2°C per 1X PCR_x Enhancer Solution concentration, while at the same time expanding the effective annealing temperature over a much broader range. To determine optimal concentrations and conditions, start with an annealing temperature of 55–60°C and vary the amount of 10X PCR_x Enhancer Solution. For targets with higher GC content (60–90%), test 10X PCR_x Enhancer Solution at final concentrations of 0.5X, 1X, 2X, and 3X.

Perform the PCR

The following protocol is specific for Platinum™ *Pfx* DNA Polymerase. For optimal performance, carefully follow these instructions.

1. Add the following components to a sterile microcentrifuge tube at room temperature or on ice.

Component	Volume for one 50- μ L reaction	Final concentration
10X <i>Pfx</i> Amplification Buffer	5–10 μ L ^[1]	1–2X
10 mM dNTP mixture	1.5 μ L	0.3 mM each
50 mM MgSO ₄	1 μ L	1 mM
Primer mix (10 μ M each)	1.5 μ L	0.3 μ M each
Template DNA (10 pg to 200 ng)	$\geq$ 1 μ L	as required
Platinum™ <i>Pfx</i> DNA Polymerase	0.4 μ L	1 U
Autoclaved, distilled water	to 50 μ L	—

^[1] Use 5 μ L (1X final concentration) of buffer as a general starting point.

Note: The volumes provided are for a single reaction. Prepare a master mix of common components for multiple reactions.

2. Mix the tube contents. Cap the tube, then centrifuge briefly to collect the contents.
3. Denature the template for 2–5 minutes at 94°C. Note that a longer denaturation time (up to 5 minutes) may increase yield and specificity.
4. Perform 25–35 cycles of PCR amplification as follows:

Step	Three-step cycling		Two-step cycling	
	Temp.	Time	Temp.	Time
Denature	94°C	15 seconds	94°C	15 seconds
Anneal	55°C	30 seconds	—	—
Extend	68°C	1 minute per kb	68°C	1 minute per kb

Note: Two-step cycling can be used for long primers with high T_m .

5. Maintain the reaction at 4°C after cycling. Samples can be stored at –20°C until use.
6. Analyze the products by agarose gel electrophoresis.

Limited product warranty

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Revision	Date	Description
A.0	5 May 2016	Format, style, and legal updates
—	11 May 2010	Baseline for this revision history

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