Platinum® *Taq* PCR$_x$ DNA Polymerase

Package Contents

Catalog Number	Size
11509-015	500 units

Kit Contents

Storage Conditions

- Store all contents at -20°C.

Required Materials

- Template: cDNA, gDNA, λDNA
- Forward and reverse gene-specific primers
- 10 mM dNTP mix (Cat. no. 18427-088)
- Autoclaved, distilled water
- E-Gel® General Purpose Gels, 1.2% (Cat. no. G5018-01)
- TrackIt™ 1 Kb Plus DNA Ladder (Cat. no. 10488-085)
- 0.2 or 0.5-mL nuclease-free microcentrifuge tubes

Timing

Varies depending on amplicon length

Selection Guide

PCR Enzymes and Master Mixes
Go online to view related products.

Product Description

- Platinum® *Taq* PCR$_x$ DNA Polymerase is recombinant *Taq*, complexed with a proprietary antibody that blocks polymerase activity at ambient temperatures, providing an automatic "hot-start" upon initial denaturation.
- An optimized buffer system is also supplied, including 10X PCR$_x$ Amplification Buffer, 50 mM $MgSO_4$, and 10X PCR$_x$ Enhancer Solution, a novel PCR cosolvent that simplifies amplification of problematic and/or GC-rich templates.

Important Guidelines

- Take precautions to avoid cross-contamination by using aerosol-resistant barrier tips and analyzing PCR products in a separate area from PCR assembly.
- Two buffers are provided: The standard 10X PCR Buffer and 50 mM $MgCl_2$ are recommended for PCR of routine templates (30–50% GC content). Substitute standard components with 10X PCR$_x$ Amplification Buffer and $MgSO_4$ for more robust amplification.
- Use 10X PCR$_x$ Enhancer with 10X PCR$_x$ Amplification Buffer and $MgSO_4$ for GC-rich templates to widen reaction optima and increase PCR success rates.
- The optimal concentration of 10X PCR$_x$ Enhancer Solution will vary depending on GC content, Mg^{++} concentration, and annealing temperature.

Online Resources

Visit our product page for additional information and protocols. For support, visit www.lifetechnologies.com/support.

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Print Options

Enzyme Characteristics

Hot-start:	Antibody
Length:	Up to 5 kb
Fidelity vs. *Taq*:	1X
Format:	Separate components

PCR Reaction Setup

Use the measurements below to prepare your PCR experiment, or enter your own parameters in the column provided.

Component	25-µL rxn	50-µL rxn	Custom	Final Conc.
Autoclaved, distilled water	to 25 µL	to 50 µL	to µL	–
10X PCR Buffer, Minus Mg	2.5 µL	5 µL	µL	1X
50 mM $MgCl_2$	0.75 µL	1.5 µL	µL	1.5 mM
10 mM dNTP Mix	0.5 µL	1 µL	µL	0.2 mM each
10 µM forward primer	0.5 µL	1 µL	µL	0.2 µM
10 µM reverse primer	0.5 µL	1 µL	µL	0.2 µM
Template DNA	varies	varies		< 500 ng
Platinum® *Taq* DNA Polymerase (5 U/µL)	0.25 µL	0.5 µL	µL	2.5 U

A. Incubate in a thermal cycler at 94°C for 2 minutes to denature the template and activate the enzyme.
B. Perform 25 to 35 cycles of PCR amplification:
   Denature—94°C for 15 seconds
   Anneal—~55°C depending on Primer T_m for 30 seconds
   Extend—72°C for 1 minute/kb

Optimization PCR Protocol for Problematic/GC-Rich

See page 2 to view a procedure for optimizing your PCR experiment by testing multiple concentrations of the PCR$_x$ Enhancer Solution in your reactions.

Optimization Strategies

Refer to the pop-up for guidelines to optimize your PCR reactions.

Limited Warranty, Disclaimer, and Licensing Information

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Platinum® *Taq* PCR$_x$ DNA Polymerase Protocol

The example PCR procedure below, for GC-rich or problematic templates, is designed to test six varying concentrations (0X to 4X) of PCR$_x$ Enhancer Solution by preparing enough master mix for seven 50-µL reactions. You can also prepare individual PCR mixtures according to the guidelines below by selecting a final concentration of PCR$_x$ Enhancer Solution to use in your reaction set-up.

Timeline	Steps	Procedure Details
1 	**Thaw reagents**	Thaw, mix, and briefly centrifuge each component before use.
2 	**Prepare PCR master mix**	Add the following components to each PCR tube. Mix and briefly centrifuge the contents. **Note:** Consider the volumes for all components listed in steps 2 and 3 to determine the correct amount of water required to reach your final reaction volume.
3 	**Add enhancer solution**	Add the following reagents to **six** microcentrifuge tubes:
4 	**Incubate reactions in a thermal cycler**	Cap each tube, mix, and then briefly centrifuge the contents.
5 	**Analyze with gel electrophoresis**	Analyze 10 µL using agarose gel electrophoresis. Use your PCR reaction immediately for down-stream applications, or store it at -20°C.

Component	Seven-Rxn Master Mix	One 50-µL Rxn	Final Concentration
Autoclaved, distilled water	to 210 µL	to 30 µL	
10X PCR$_x$ Amplification Buffer	35 µL	5 µL	1X
10 mM dNTP Mixture	7 µL	1 µL	0.2 mM each
50 mM $MgSO_4$	10.5 µL	1.5 µL	1.5 mM
10 µM Forward Primer	7 µL	1 µL	0.2 µM
10 µM Reverse Primer	7 µL	1 µL	0.2 µM
Template DNA	≥ 7 µL	≥ 1 µL	< 500 ng
Platinum® *Taq* DNA Polymerase (5 U/µL)	3.5 µL	0.5 µL	2.5 U/rxn

Component	PCRx Enhancer Concentration					
	0X	**0.5X**	**1X**	**2X**	**3X**	**4X**
Master Mix from step 2	30	30	30	30	30	30
10X PCR$_x$ Enhancer Solution	-	2.5	5	10	15	20
Autoclaved, distilled water	20	17.5	15	10	5	-

Step		Temperature (°C)	Time
Initial Denaturation		95	2 minutes
25–35 PCR Cycles	Denature	95	45 seconds
	Anneal	~55 (depending on primer T_m)	30 seconds
	Extend	68	1 minute/kb
Hold		4	indefinitely