Invitrogen™ Platinum™ II Hot-Start Green PCR Master Mix (2X)

invitrogen

USER GUIDE

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Print Options

Package contents

Catalog No.	Size
14001-012	50 reactions
14001-013	200 reactions
14001-014	1000 reactions

Kit contents

Storage conditions

All the components of the kit can be stored at 4°C for periods up to 3 months. For longer storage, keep all components at –20°C.

Required materials

- Template: cDNA, genomic DNA, plasmid DNA, phage DNA
- Forward and reverse primers
- Invitrogen™ E-Gel™ EX Agarose Gels, 1% (Cat. No. G4010-01)
- Invitrogen™ E-Gel™ 1 kb Plus Express DNA Ladder (Cat. No. 10488-091)
- 0.2 or 0.5-mL nuclease-free microcentrifuge tubes

Timing

Varies depending on amplicon length.

Product description

- Invitrogen™ Platinum™ II Hot-Start Green PCR Master Mix (2X) contains Platinum™ II *Taq* Hot-Start DNA Polymerase premixed in an optimized Platinum™ II PCR buffer with dNTPs. The master mix is supplemented with tracking dyes for direct loading of PCR products on gels.
- Platinum™ II *Taq* Hot-Start DNA Polymerase is an engineered *Taq* DNA polymerase that shows increased resistance to reaction inhibitors originating from sample material or DNA purification steps.
- The polymerase activity is blocked at ambient temperatures and restored after the initial denaturation step at 94°C. This automatic "hot start" provides increased sensitivity, specificity, and yield, while allowing reaction assembly at room temperature.
- Due to unique composition of the Platinum™ II PCR buffer, the annealing temperature is 60°C for most primer pairs designed following general primer design rules.
- Platinum™ II *Taq* DNA polymerase extends 1 kb in 15 seconds. The extension step can be prolonged without a negative effect on specificity.
- The enzyme has a template independent terminal transferase activity that adds a single deoxyadenosine (A) to the 3′ ends of PCR products. Like standard *Taq*, it has both 5′ to 3′ polymerase and 5′ to 3′ exonuclease activities, but lacks 3′ to 5′ exonuclease activity.
- The tracking dyes (a blue dye and a yellow dye) in the master mix do not interfere with PCR performance and are compatible with downstream applications such as ligation or restriction digestion.

Online resources

Visit our product page for additional information.
Find out more at thermofisher.com/platinumiitaq.
For support, visit thermofisher.com/support.

Enzyme characteristics

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

PCR setup

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

Component	20-µL rxn	50-µL rxn	Custom	Final conc.
Water, nuclease-free	to 20 µL	to 50 µL	to µL	—
Platinum™ II Hot-Start Green PCR Master Mix (2X)[1]	10 µL	25 µL	µL	1X
10 µM forward primer	0.4 µL	1 µL	µL	0.2 µM
10 µM reverse primer	0.4 µL	1 µL	µL	0.2 µM
Template DNA[2]	varies	varies	µL	<500 ng/rxn
Platinum™ GC Enhancer (*optional*)[3]	4 µL	10 µL	µL	1X

[1] Provides 1.5 mM $MgCl_2$ in final reaction concentration.
[2] 0.5–500 ng genomic DNA, 1 pg–50 ng plasmid or viral DNA, or 1–5 µL of cDNA synthesis reaction per 50-µL PCR reaction.
[3] Recommended for targets with >65% GC sequences.

PCR protocol

Go to page 2 for instructions to prepare and run your PCR experiment.

Important guidelines

Click here for important PCR guidelines.

Optimization strategies

Click here for guidelines to optimize your PCR experiment.

Troubleshooting

Click here to troubleshoot your PCR experiment.

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PCR protocol

The example procedure below shows the appropriate volumes for a single **50-µL** reaction. For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, then dispense the appropriate volumes into each 0.2-mL or 0.5-mL PCR tube before adding template DNA and primers.

<table>
<tr><th>Steps</th><th>Action</th><th>Procedure details</th></tr>
<tr><td>1  </td><td>Thaw reagents</td><td>Thaw, mix, and briefly centrifuge each component before use.</td></tr>
<tr><td>2  </td><td>Prepare PCR master mix</td><td>a. Add the following components to each reaction tube.
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.
<table>
<tr><th>Component</th><th>Volume for 50-µL rxn</th><th>Final concentration</th></tr>
<tr><td>Water, nuclease-free</td><td>to 50 µL</td><td>—</td></tr>
<tr><td>Platinum™ II Hot-Start Green PCR Master Mix (2X)</td><td>25 µL</td><td>1X</td></tr>
<tr><td>Platinum™ GC Enhancer (optional)[1]</td><td>10 µL</td><td>1X</td></tr>
</table>
[1] Recommended for targets with >65% GC sequences.
b. Mix, then briefly centrifuge the components.</td></tr>
<tr><td>3  </td><td>Add template DNA and primers</td><td>a. Add your template DNA and primers to each tube for a final reaction volume of 50 µL.
<table>
<tr><th>Component</th><th>Volume for 50-µL rxn</th><th>Final concentration</th></tr>
<tr><td>10 µM forward primer</td><td>1 µL</td><td>0.2 µM</td></tr>
<tr><td>10 µM reverse primer</td><td>1 µL</td><td>0.2 µM</td></tr>
<tr><td>Template DNA</td><td>varies</td><td><500 ng/rxn</td></tr>
</table>
b. Cap each tube, mix, then briefly centrifuge the contents.</td></tr>
</table>

Steps	Action	Procedure details
4 	**Incubate reactions in a thermal cycler**	See table below.
5 	**Analyze with gel electrophoresis**	**a.** Analyze the sample using agarose gel electrophoresis. **Note:** Dilute the PCR sample 2- to 20-fold for optimal separation on E-Gel™ agarose gels. **b.** Use your PCR product immediately in down-stream applications, or store it at –20°C.

Step		3-step protocol		2-step protocol[1]	
		Temperature	Time	Temperature	Time
Initial denaturation		94°C	2 minutes	94°C	2 minutes
25–35 PCR cycles	Denature	94°C	15 seconds	98°C	5 seconds
	Anneal[2]	60°C	15 seconds	60°C	15 seconds
	Extend	68°C	15 seconds/kb		
Hold		4°C	hold	4°C	hold

[1] Recommended for simple amplicons up to 1 kb with 45–65% GC sequences. For longer, GC-rich, and complex amplicons, or cDNA targets, use the 3-step cycling protocol.

[2] 60°C annealing temperature works for most primers. In cases when annealing temperature requires additional optimization, we recommend performing gradient PCR or redesigning the primers.

Note: Refer to "Optimization strategies", page 1, for guidelines to optimize cycling conditions.