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

invitrogen

USER GUIDE

Pub. No. MAN0017535 Rev. A.0

Print Options

## Package contents

| Catalog No. | Size |
|---|---|
| 14000-012 | 50 reactions |
| 14000-013 | 200 reactions |
| 14000-014 | 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
- Gel loading buffer

## Timing

Varies depending on amplicon length.

## Product description

- Invitrogen™ Platinum™ II Hot-Start PCR Master Mix (2X) contains Platinum™ II *Taq* Hot-Start DNA Polymerase premixed in an optimized Platinum™ II PCR buffer with dNTPs.
- 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.

## Online resources

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

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

## 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 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.

## Limited warranty, disclaimer, and licensing information

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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.<br>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 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.<br>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 |
|---|---|---|

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**Incubate reactions in a thermal cycler**

| 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 rdesigning the primers.

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

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**Add gel loading buffer and analyze with gel electrophoresis**

**a.** Add gel loading buffer to 10 µL of PCR sample, mix, and briefly centrifuge the contents.

**Note:** Dilute the PCR sample 2- to 20-fold for optimal separation on E-Gel™ agarose gels.

**b.** Analyze the sample using agarose gel electrophoresis.

**c.** Use your PCR product immediately in down-stream applications, or store it at –20°C.