Invitrogen™ Platinum™ SuperFi™ II PCR Master Mix

USER GUIDE Pub. No. MAN0018860 Rev. B.0

invitrogen

Package contents

Catalog number	Size
12368010	100 reactions
12368050	500 reactions
12368250	5 × 500 reactions

Kit contents

Storage conditions

- Store all contents at –20°C.

Required materials

Click here for required materials

Product description

- Platinum™ SuperFi™ II DNA Polymerase is a proofreading DNA polymerase that combines high fidelity with Platinum™ hot-start technology and universal primer annealing. It is ideal for cloning, mutagenesis, and other applications.
- Platinum™ SuperFi™ II PCR Master Mix is a ready-to-use mixture of DNA polymerase, salts, magnesium, and dNTPs for efficient PCR amplification, which retains all the features of the Platinum™ SuperFi™ II DNA Polymerase.
- The annealing temperature with Platinum™ SuperFi™ II DNA Polymerase is 60°C. Proprietary additives in the reaction buffer stabilize primer-template duplexes during the annealing step, and contribute to increased specificity without the need to optimize annealing temperature for each primer pair.
- Due to proprietary additives in the reaction buffer, Platinum™ SuperFi™ II DNA Polymerase shows efficient amplification of both AT and GC rich targets. Additional DNA melting agents are not required for GC-rich PCR (up to 75% GC).
- Platinum™ hot-start technology inhibits DNA polymerase activity at ambient temperatures, allowing room temperature reaction setup and storage of pre-assembled PCR reactions. Enzyme activity is restored after the initial denaturation step.
- Platinum™ SuperFi™ II DNA Polymerase has 5' to 3' polymerase and 3' to 5' exonuclease activities, but lacks 5' to 3' exonuclease activity. It produces blunt end DNA products.

Selection guide

PCR Enzymes and Master Mixes

Go online to view related products.

Online resources

Visit our product page for additional information and protocols.

For support, visit thermofisher.com/support.

Enzyme characteristics

Hot-start:	Antibody
Length:	Up to 20 kb
Fidelity vs. *Taq*:	>300X
Timing:	Varies depending on amplicon length
Format:	Master Mix

PCR setup

Component	Final concentration	20-µL rxn	50-µL rxn
2X Platinum™ SuperFi™ II PCR Master Mix [1]	1X	10 µL	25 µL
Forward primer	0.5 µM [2]	*x* µL	*x* µL
Reverse primer	0.5 µM [2]	*x* µL	*x* µL
Template DNA	0.1–10 ng plasmid (5–100 ng genomic DNA)	*x* µL	*x* µL
Water, nuclease-free	—	to 20 µL	to 50 µL

[1] Provides 1.75 mM $MgCl_2$ in 1X concentration.

[2] Reduce the primer concentration to 0.2 µM final for amplification of >5 kb targets from genomic DNA and for multiplex reactions.

PCR protocol

See page 2 to prepare and run your PCR experiment.

Important guidelines

Click here for Important guidelines.

Optimization strategies and troubleshooting

Click here for Optimization strategies for your PCR experiment.

Click here for Troubleshooting for your PCR experiment.

Purchaser notification

Click here for Limited product warranty, Disclaimer, and Important licensing information.

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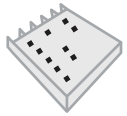
Prepare and run PCR

The example PCR procedure below shows appropriate volumes for a single **20-µL** or **50-µL** reaction. For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, then dispense 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 reaction mix with template DNA and primers</td><td>Add the following components to each PCR tube.
Note: Consider the volumes for all components in reaction mix to determine the correct amount of water required to reach your final reaction volume.
<table>
<tr><th>Component</th><th>Final concentration</th><th>20-µL rxn</th><th>50-µL rxn</th></tr>
<tr><td>2X Platinum™ SuperFi™ II PCR Master Mix [1]</td><td>1X</td><td>10 µL</td><td>25 µL</td></tr>
<tr><td>Forward primer</td><td>0.5 µM [2]</td><td>x µL</td><td>x µL</td></tr>
<tr><td>Reverse primer</td><td>0.5 µM [2]</td><td>x µL</td><td>x µL</td></tr>
<tr><td>Template DNA</td><td>0.1–10 ng plasmid
(5–100 ng genomic DNA)</td><td>x µL</td><td>x µL</td></tr>
<tr><td>Water, nuclease-free</td><td>—</td><td>to 20 µL</td><td>to 50 µL</td></tr>
</table>
[1] Provides 1.75 mM $MgCl_2$ in 1X concentration.
[2] Reduce the primer concentration to 0.2 µM final for amplification of >5 kb targets from genomic DNA and for multiplex reactions.

Cap each tube, mix, and then briefly centrifuge the contents.</td></tr>
<tr><td>3  </td><td>Incubate reactions in a thermal cycler
(3-step protocol)</td><td>3-step protocol
<table>
<tr><th>Cycle step</th><th>Temperature</th><th>Time</th><th>Cycles</th></tr>
<tr><td>Initial denaturation</td><td>98°C</td><td>30 seconds</td><td>1</td></tr>
<tr><td>Denaturation
Annealing
Extension</td><td>98°C
60°C
72°C</td><td>5–10 seconds
10 seconds
15–30 seconds per 1 kb</td><td>25–35</td></tr>
<tr><td>Final extension</td><td>72°C
4°C</td><td>5 minutes
Hold</td><td>1
—</td></tr>
</table>
Note: Refer to "Important guidelines", page 1, for guidelines to optimize cycling conditions.</td></tr>
</table>

<table>
<tr><th>Steps</th><th>Action</th><th>Procedure details</th></tr>
<tr>
<td>3  </td>
<td>Incubate reactions in a thermal cycler
(2-step protocol)</td>
<td>
2-step protocol (for primers >30 nt long)[1]
<table>
<tr><th>Cycle step</th><th>Temperature</th><th>Time</th><th>Cycles</th></tr>
<tr><td>Initial denaturation</td><td>98°C</td><td>30 seconds</td><td>1</td></tr>
<tr><td>Denaturation
Annealing/Extension</td><td>98°C
72°C</td><td>5–10 seconds
15–30 seconds per 1 kb</td><td>25–35</td></tr>
<tr><td>Final extension</td><td>72°C
4°C</td><td>5 minutes
hold</td><td>1
—</td></tr>
</table>
[1] Without non-complementary parts (e.g. restriction tags).
Note: Refer to "Important guidelines", page 1, for guidelines to optimize cycling conditions.
</td>
</tr>
<tr>
<td>4  </td>
<td>Add gel loading buffer and analyze with gel electrophoresis</td>
<td>
Add gel loading buffer to 10 µL of PCR product, mix, and briefly centrifuge the contents.
Note: For optimal separation using E-Gel™ agarose gels, dilute the sample 2- to 20-fold.
Analyze the sample using agarose gel electrophoresis.
Use your PCR product immediately in down-stream applications, or store it at –20°C.
</td>
</tr>
</table>

Kit contents

Reagents provided are sufficient for 100, 500, or 2500 amplification reactions of 50 µL each.

	Kit sizes		
Component	100 reactions	500 reactions	2500 reactions
Platinum™ SuperFi™ II PCR Master Mix	2 × 1.25 mL	10 × 1.25 mL	50 × 1.25 mL
Water, nuclease-free	2 × 1.25 mL	10 × 1.25 mL	50 × 1.25 mL
Water, nuclease-free	2 × 1.25 mL	10 × 1.25 mL	50 × 1.25 mL

Required materials

- Template: genomic DNA, plasmid DNA, phage DNA, cDNA
- Forward and reverse primers
- Invitrogen™ E-Gel™ Agarose Gels with SYBR™ Safe DNA Stain, 1.2% (Cat. No. G521801)
- Invitrogen™ E-Gel™ 1 kb Plus DNA Ladder (Cat. No. 10488090)
- 0.2-mL or 0.5-mL nuclease-free microcentrifuge tubes

Important guidelines

- Platinum™ SuperFi™ II DNA Polymerase cannot read dUTP-derivatives or dITP in the template strand. Therefore, primers and dNTP mixes containing such nucleotides are not compatible.
- Carefully mix and centrifuge all tubes before opening to ensure homogeneity and to improve recovery. Prepare a master mix for the appropriate number of samples to be amplified.
- When using Platinum™ SuperFi™ II DNA Polymerase, it is not necessary to perform the PCR set up on ice.
- Take precautions to avoid cross-contamination by using aerosol-resistant barrier tips and by analyzing PCR products in a separate area from PCR assembly.

Optimization strategies

Reaction components

Primers

- Design 18- to 35-mers with 40–60% GC content. If possible, design the primers with one or two G or C bases at the 3′ end. Avoid primer pairs with complementarity at 3′ ends or >10°C melting temperature (T_m) difference.
- Verify primer complementarity to a single template region using programs for sequence alignment. Online primer design programs such as the Invitrogen™ OligoPerfect™ Designer can be helpful.
- We recommend a final primer concentration of 0.5 µM, but this can be varied in a range of 0.1–1.0 µM, if needed. For amplification of >5 kb targets from high complexity DNA and for multiplex reactions, we recommend lower primer concentrations (0.2 µM final).

Template

- Low complexity DNA: Optimal amount of low complexity DNA (plasmid, phage or BAC DNA) is 0.1–10 ng per 50 µL reaction, but it can be varied from 0.1 pg to 50 ng per 50 µL reaction. For long targets, we recommend using higher amounts of template.
- Genomic DNA: Optimal amount of genomic DNA is 5–100 ng per 50 µL reaction, but it can be varied from 0.1 ng to 250 ng per 50 µL reaction. We recommend higher template amount for long targets.
- cDNA: Optimal amount of cDNA is 0.1–1 µL of the first-strand reaction mixture per 50 µL reaction.

Optional reaction components

- **Mg^{2+}: Platinum™** SuperFi™ II Reaction Buffer provides $MgCl_2$ at a final concentration of 1.75 mM in the reaction. If the primers and/or the template contain chelators such as EDTA or EGTA, the apparent Mg2+ optimum may be shifted to higher concentrations.
- **DMSO:** Platinum™ SuperFi™ II DNA Polymerase can amplify targets with high GC content (up to 75% GC) without any additional DNA melting agents. In cases of extremely GC-rich targets (>75% GC), we recommend addition of DMSO to a final concentration of 5%.

Cycling parameters

Number of cycles

Total amount of PCR cycles can vary from 15 to 40, depending on target length and template amount. For low complexity templates 25–30 PCR cycles is typical; 30–35 cycles are recommended for genomic DNA.

Denaturation

- Use 98°C for denaturation. Ensure that the heated lid temperature is set several degrees above 98°C to avoid sample condensation.
- 30-second initial denaturation at 98°C is sufficient for most templates. You can extend the initial denaturation time up to 5 minutes, if needed.

Annealing

- Due to unique isostabilizing molecules in the Platinum™ SuperFi™ II Reaction Buffer, 60°C annealing temperature works for most primers.
- We recommend the 2-step protocol when primers without non-complementary parts are >30 nt in length (e.g. primers for site-specific mutagenesis). In the 2-step protocol, perform the combined annealing/extension step at 72°C.
- If the amplification does not give satisfactory results, we recommend using a temperature gradient. The annealing temperature can be optimized using Applied Biosystems™ thermal cyclers, such as the ProFlex™ PCR System or the Veriti™ Thermal Cycler featuring VeriFlex™ technology.

Extension

- Extension time depends on amplicon length and complexity. For low complexity DNA (e.g. plasmid, lambda or BAC DNA), use an extension time of 15 seconds per 1 kb. For high complexity genomic DNA, use an extension time of 30 seconds per 1 kb.
- You can prolong the extension step up to 90 seconds per 1 kb for targets up to 5 kb without negative effect on specificity. Prolonged extention time allows the amplification of shorter and longer amplicons together using the same protocol.

Troubleshooting

Observation	Recommended action
No product at all or low yield.	▪ Repeat the PCR and make sure that there are no pipetting errors. ▪ Do not use dNTP mix or primers that contain dUTP or dITP. ▪ Check primer design and concentration. ▪ Run a temperature gradient to determine optimal annealing temperature. ▪ Increase the total number of cycles. ▪ Titrate the template amount. Too little or too much template can compromise PCR results.
Non specific products or smears	▪ Run a temperature gradient to determine optimal annealing temperature. ▪ Decrease extension time. ▪ Reduce the total number of cycles. ▪ Reduce the primer concentration.

Limited product warranty

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