Invitrogen™ Platinum™ SuperFi™ Green PCR Master Mix

invitrogen

USER GUIDE Pub. No. MAN0014885 Rev. B

Print Options

Package contents

Catalog number	Size
12359-010	100 rxns
12359-050	500 rxns
12359-250	5 × 500 rxns

Kit contents

Storage conditions

- Store all contents at –20°C.

Required materials

- Template: gDNA, plasmid DNA, phage DNA, cDNA
- Forward and reverse gene-specific primers
- Invitrogen™ E-Gel™ General Purpose Gels, 1.2% (Cat. No. G501801)
- Invitrogen™ TrackIt™ 1 kb Plus DNA Ladder (Cat. No. 10488085)
- 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

- Invitrogen™ Platinum™ SuperFi™ Green PCR Master Mix is a ready-to-use mixture of DNA polymerase, salts, magnesium, and dNTPs for efficient PCR amplification,while retaining all the features of the Platinum™ SuperFi™ DNA Polymerase. In addition, it is supplemented with two tracking dyes and a density reagent for direct loading of PCR products on gels.
- Platinum™ SuperFi™ DNA Polymerase is a proofreading DNA polymerase that combines fidelity with Platinum™ hot-start technology, and is ideally suited for cloning, mutagenesis, and other applications.
- Platinum™ hot-start technology inhibits DNA polymerase activity at ambient temperatures, allowing room temperature reaction setup and storage of pre-assembled PCR reactions for up to 24 hours prior to the PCR. Enzyme activity is restored after the initial denaturation step.
- Platinum™ SuperFi™ 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.
- Platinum™ SuperFi™ PCR Master Mix is supplied with a separate vial of SuperFi™ GC Enhancer designed for GC-rich templates (>65% GC).

Important guidelines

Click here for important PCR guidelines.

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*:	>100X
Format:	Master Mix

PCR setup

Component	25-µL rxn	50-µL rxn	Custom	Final conc.
Water, nuclease-free	to 25 µL	to 50 µL	to µL	—
2X Platinum™ SuperFi™ Green PCR Master Mix[1]	12.5 µL	25 µL	µL	1X
10 µM forward primer	1.25 µL	2.5 µL	µL	0.5 µM
10 µM reverse primer	1.25 µL	2.5 µL	µL	0.5 µM
Template DNA[2]	varies	varies		varies
5X SuperFi™ GC Enhancer (*optional*)[3]	5 µL	10 µL	µL	1X

[1] Provides $MgCl_2$ at a final concentration of 1.5 mM in the reaction.
[2] 5–50 ng gDNA or 1 pg–10 ng plasmid DNA (see "**Optimization strategies**", below, for more information).
[3] Recommended for targets with >65% GC sequences.

PCR protocol

See page 2 and page 3 to prepare and run your PCR experiment.

Optimization strategies and troubleshooting

Click here for guidelines to optimize your PCR experiment.

Click here for guidelines to troubleshoot your PCR experiment.

Purchaser notification

Click here for Limited warranty, Disclaimer, and Licensing information.

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The example PCR procedure below shows 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 appropriate volumes into each 0.2–0.5 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. Avoid generating bubbles when mixing the Master Mix.</td></tr>
<tr><td>2  </td><td>Prepare PCR master mix</td><td>Add the following components to each PCR 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>50-µL rxn</th><th>Final conc.</th></tr>
<tr><td>Water, nuclease-free</td><td>to 50 µL</td><td></td></tr>
<tr><td>2X Platinum™ SuperFi™ Green PCR Master Mix</td><td>25 µL</td><td>1X</td></tr>
<tr><td>5X SuperFi™ GC Enhancer (optional)[1]</td><td>10 µL</td><td>1X</td></tr>
</table>
[1] Recommended for targets with >65% GC sequences.
Mix and then briefly centrifuge the components.</td></tr>
<tr><td>3  </td><td>Add template DNA and primers</td><td>Add your template DNA and primers to each tube for a final reaction volume of 50 µL.
<table>
<tr><th>Component</th><th>50-µL rxn</th><th>Final conc.</th></tr>
<tr><td>10 µM forward primer</td><td>2.5 µL</td><td>0.5 µM</td></tr>
<tr><td>10 µM reverse primer</td><td>2.5 µL</td><td>0.5 µM</td></tr>
<tr><td>Template DNA[1]</td><td>varies</td><td>varies</td></tr>
</table>
[1] Optimal amount of low complexity DNA (plasmid, phage, BAC DNA) is 1 pg–10 ng per 50 µL reaction, but it can be varied from 0.1 pg to 50 ng per 50 µL reaction. Optimal amount of genomic DNA is 5–50 ng per 50 µL reaction, but it can be varied from 0.1 ng to 250 ng per 50 µL reaction.
Cap each tube, mix, and 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**	Analyze the sample using agarose gel electrophoresis. **Note:** PCR mixes prepared using the SuperFi™ Green Buffer are ready for direct loading on the gels; addition of loading buffer is not needed. Use your PCR product immediately in down-stream applications, or store it at –20°C.

Step		2-step protocol (<10kb)		3-step protocol (<10kb)		Long PCR (>10kb)	
		Temp.	Time	Temp.	Time	Temp.	Time
Initial denaturation		98°C	30 sec	98°C	30 sec	95°C	2 min
25–35 PCR cycles	Denature	98°C	5–10 sec	98°C	5–10 sec	95°C	10 sec
	Anneal[1]	—	—	varies	10 sec	varies	10 sec
	Extend	72°C	15–30 sec/kb	72°C	15–30 sec/kb	68°C	30 sec/kb
Final extension		72°C	5 min	72°C	5 min	68°C	5 min
		4°C	hold	4°C	hold	4°C	hold

[1] **IMPORTANT!** Always use T_m calculator on our website at www.thermofisher.com/tmcalculator to calculate T_m of your primers and the recommended annealing temperature.

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