Invitrogen™ Platinum™ SuperFi™ PCR Master Mix

invitrogen

USER GUIDE Pub. No. MAN0014883 Rev. B

Print Options

Package contents

Catalog number	Size
12358-010	100 rxns
12358-050	500 rxns
12358-250	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. G5018-01)
- Invitrogen™ TrackIt™ 1 kb Plus DNA Ladder (Cat. No. 10488-085)
- 0.2 or 0.5-mL nuclease-free microcentrifuge tubes
- Gel loading buffer

Timing

Varies depending on amplicon length

Selection guide

PCR Enzymes and Master Mixes

Go online to view related products.

Product description

- Invitrogen™ Platinum™ SuperFi™ PCR Master Mix is a ready-to-use mixture of DNA polymerase, salts, magnesium, and dNTPs for efficient PCR amplification. It retains all the features of the Platinum™ SuperFi™ DNA Polymerase.
- 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 both 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™ 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** 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

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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 prior to 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™ 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 	**Add gel loading buffer and analyze with gel electrophoresis**	Add gel loading buffer to 10 µL of PCR product, mix, and briefly centrifuge the contents. Analyze the sample using agarose gel electrophoresis. 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.

Package contents

Catalog numbe
12358-010
12358-050
12358-250

Storage conditions

- Store all cont

Required materials

- Template: gD
- Forward and
- Invitrogen™ P (Cat. No. G50
- Invitrogen™ T (Cat. No. 10488-085)

Timing

Selecti guide

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- 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

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Kit contents

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

	Kit sizes		
Component	**100 reactions**	**500 reactions**	**2500 reactions**
2X Platinum™ SuperFi™ PCR Master Mix	2 × 1.25 mL	10 × 1.25 mL	50 × 1.25 mL
5X SuperFi™ GC Enhancer	1.25 mL	4 × 1.25 mL	20 × 1.25 mL
Water, nuclease-free	2 × 1.25 mL	10 × 1.25 mL	50 × 1.25 mL

Important guidelines

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- The annealing rules for Invitrogen™ Platinum™ SuperFi™ DNA Polymerase are different from many common DNA polymerases (such as *Taq* DNA polymerases). **For optimal results, use the T_m calculator on our website at www.thermofisher.com/tmcalculator.**
- 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™ PCR Master Mix, it is not necessary to perform the PCR set up on ice.
- Pipet the Platinum™ SuperFi™ PCR Master Mix carefully and gently. Otherwise, the high glycerol content (50%) in the storage buffer may lead to pipetting errors.
- Platinum™ SuperFi™ DNA Polymerase produces blunt end DNA products.
- The polymerase cannot read dUTP-derivatives or dITP in the template strand. Therefore, we do not recommend the use of these analogues or primers that contain them.
- Take precautions to avoid cross-contamination by using aerosol-resistant barrier tips and by analyzing PCR products in a separate area from PCR assembly.
- Use 15–30 seconds/kb for extension. Do not exceed 1 minute/kb.
- Use the SuperFi™ GC Enhancer to improve amplification of DNA targets containing GC-rich sequences (>65% GC).
- For a streamlined protocol, we recommend using Invitrogen™ Platinum™ SuperFi™ Green PCR Master Mix (Cat. No. 12359-010), which contains tracking dyes and a density reagent for direct loading of PCR products on gels.

rxn	Custom	Final conc.
µL	to µL	—
		1X
		0.5 µM
		0.5 µM
		varies
		1X

more

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Optimization strategies

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Notes about reaction components

Polymerase: Optimal amount of Platinum™ SuperFi™ DNA Polymerase is 1 U per 50 µL reaction in most cases, but it can be varied in a range of 0.5–2.0 U per 50 µL reaction.

Do not exceed 2 U of polymerase per 50 µL reaction (0.04 U/µL).

Mg^{2+}: SuperFi™ Buffer provides $MgCl_2$ at a final concentration of 1.5 mM in the reaction.

- If the primers and/or the template contain chelators such as EDTA or EGTA, the apparent Mg^{2+} optimum may be shifted to higher concentrations.
- If further optimization is needed, increase the Mg^{2+} concentration in 0.2 mM increments.

dNTPs: Use high quality dNTPs for optimal performance and highest fidelity.

- The polymerase cannot read dUTP derivatives or dITP in the template strand. Therefore, we do not recommend the use of these analogues or primers that contain them.
- Due to high processivity of the enzyme, there is no advantage of increasing the dNTP concentration. For optimal results, always use 200 µM of each dNTP.

Primers: We recommend a final primer concentration of 0.5 µM, but this can be varied in a range of 0.2–1.0 µM, if needed.

Template: 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.

SuperFi™ GC Enhancer: Use the SuperFi™ GC Enhancer to improve amplification of DNA targets containing problematic or GC-rich sequences (>65% GC).

Notes about cycling parameters

Make sure that the heated lid temperature is set several degrees above 98°C to avoid sample condensation. The lid can be pre-heated before putting the samples in the thermocycler.

Initial denaturation: 30-second initial denaturation at 98°C is sufficient for most templates. Some templates may require longer initial denaturation time, which can be extended up to 3 minutes. For targets >10 kb, we recommend an initial denaturation of 2 minutes at 95 °C.

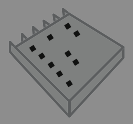
Denaturation: Keep the denaturation time as short as possible. Usually 5–10 seconds at 98°C is sufficient for most templates. For targets >10 kb, reduce the denaturation temperature to 95°C.

Annealing: Always use the T_m calculator available on our website (www.thermofisher.com/tmcalculator) to calculate the T_m of your primers and the recommended annealing temperature.

- If necessary, use a temperature gradient to further optimize and empirically determine the ideal annealing temperature for each template-primer pair combination. The annealing gradient should start with a temperature 6–10°C lower than the annealing temperature generated by the calculator and increased up to the extension temperature (two-step PCR).
- We recommend a 2-step protocol when the primer T_m values are at least 69°C (>20 nt) or 72°C (≤20 nt), when calculated with our T_m calculator. In the 2-step protocol, the combined annealing/extension step should be performed at 72°C even when the primer T_m is >72°C.

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.
- For some cDNA templates, the extension time can be increased up to 40 seconds per 1 kb to obtain optimal results.
- Reduce the extension temperature to 68°C for targets >10 kb.

Steps		
4		Incubate ther
5		Add gel lo analy elect

	Long PCR (>10kb)	
	Temp.	Time
	95°C	2 min
	95°C	10 sec
	varies	10 sec
kb	68°C	30 sec/kb
	68°C	5 min
	4°C	hold

mcalculator to calculate T_m of your

ycling conditions.

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t –20°C.

Troubleshooting

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Observation	Recommended action
No product at all or low yield.	▪ Make sure that there are no pipetting errors. ▪ Do not use primers or template that contain dUTP or dITP. ▪ Titrate template amount. ▪ Template DNA may be damaged. Use carefully purified template. ▪ Increase extension time. ▪ Increase the number of cycles. ▪ Decrease annealing temperature. ▪ Optimize denaturation time and temperature. ▪ Check the purity and concentration of the primers. ▪ Check primer design. ▪ Try using the SuperFi™ GC Enhancer
Non specific products–High molecular weight smears	▪ Decrease extension time. ▪ Reduce the total number of cycles. ▪ Increase annealing temperature or try 2-step protocol. ▪ Optimize denaturation time and temperature. ▪ Reduce enzyme concentration. ▪ Reduce primer concentration. ▪ Check primer design
Non specific products–Low molecular weight smears	▪ Increase annealing temperature. ▪ Decrease enzyme concentration. ▪ Decrease extension time. ▪ Titrate template amount. ▪ Decrease primer concentration. ▪ Check primer design