

	Catalog number	Size	
Package contents	12361010	100 reactions	Kit contents
	12361050	500 reactions	
	12361250	5 × 500 reactions	

Storage conditions	
	<ul style="list-style-type: none"> Store all contents at -20°C.

Required materials	
	<ul style="list-style-type: none"> Template: genomic DNA, plasmid DNA, phage DNA, cDNA Forward and reverse primers Invitrogen™ 10 mM dNTP Mix (Cat. No. 18427-088) 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 Water, nuclease-free

Product description	
	<ul style="list-style-type: none"> 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 ideally suited for cloning, mutagenesis, and other applications. 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. <i>Taq</i>:	>300X
Timing:	Varies depending on amplicon length
Format:	Separate components

PCR setup

Component	Final concentration	20- μL rxn	50- μL rxn
5X SuperFi™ II Buffer ^[1]	1X	4 μL	10 μL
Forward primer	0.5 μM ^[2]	X μL	X μL
Reverse primer	0.5 μM ^[2]	X μL	X μL
10 mM dNTPs	200 μM each	0.4 μL	1 μL
Template DNA	0.1–10 ng plasmid DNA (5–100 ng genomic DNA)	X μL	X μL
Platinum™ SuperFi™ II DNA Polymerase	1X	0.4 μL	1 μL
Water, nuclease-free	—	to 20 μL	to 50 μL

^[1] Provides 1.5 mM MgCl_2 in 1X concentration.

^[2] Reduce 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 and page 3 to prepare and run your PCR experiment.

Important guidelines

Click here for important PCR guidelines.

Optimization strategies and troubleshooting

Click here for guidelines to optimize your PCR experiment.


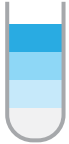

Click here for guidelines to troubleshoot your PCR experiment.

Purchaser notification


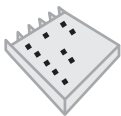
Click here for Limited Warranty, Disclaimer, and Licensing information.

Prepare PCR

The example PCR procedure below shows appropriate volumes for a single **20- μ L** or **50- μ L** 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.

Steps	Action	Procedure details																				
1 	Thaw reagents	Thaw, mix, and briefly centrifuge each component before use.																				
2 	Prepare PCR master mix	<p>Add the following components to each PCR tube.</p> <p>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.</p> <table border="1"> <thead> <tr> <th>Component</th> <th>Final concentration</th> <th>20-μL rxn</th> <th>50-μL rxn</th> </tr> </thead> <tbody> <tr> <td>Water, nuclease-free</td> <td>—</td> <td>to 20 μL</td> <td>to 50 μL</td> </tr> <tr> <td>5X SuperFi™ II Buffer^[1]</td> <td>1X</td> <td>4 μL</td> <td>10 μL</td> </tr> <tr> <td>10 mM dNTPs</td> <td>200 μM each</td> <td>0.4 μL</td> <td>1 μL</td> </tr> <tr> <td>Platinum™ SuperFi™ II DNA Polymerase</td> <td>—</td> <td>0.4 μL</td> <td>1 μL</td> </tr> </tbody> </table> <p>^[1] Includes 7.5 mM MgCl₂.</p> <p>Mix and then briefly centrifuge the components.</p>	Component	Final concentration	20- μ L rxn	50- μ L rxn	Water, nuclease-free	—	to 20 μ L	to 50 μ L	5X SuperFi™ II Buffer ^[1]	1X	4 μ L	10 μ L	10 mM dNTPs	200 μ M each	0.4 μ L	1 μ L	Platinum™ SuperFi™ II DNA Polymerase	—	0.4 μ L	1 μ L
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3 	Add template DNA and primers	<p>Add your template DNA and primers to each tube for a final reaction volume of 20 μL or 50 μL.</p> <table border="1"> <thead> <tr> <th>Component</th> <th>Final concentration</th> <th>20-μL rxn</th> <th>50-μL rxn</th> </tr> </thead> <tbody> <tr> <td>10 μM forward primer</td> <td>0.5 μM^[1]</td> <td>X μL</td> <td>X μL</td> </tr> <tr> <td>10 μM reverse primer</td> <td>0.5 μM^[1]</td> <td>X μL</td> <td>X μL</td> </tr> <tr> <td>Template DNA</td> <td>0.1–10 ng plasmid DNA (5–100 ng genomic DNA)</td> <td>X μL</td> <td>X μL</td> </tr> </tbody> </table> <p>^[1] Reduce the primer concentration to 0.2 μM final for mplification of >5 kb targets from genomic DNA and for multiplex reactions.</p> <p>Cap each tube, mix, and then briefly centrifuge the contents.</p>	Component	Final concentration	20- μ L rxn	50- μ L rxn	10 μ M forward primer	0.5 μ M ^[1]	X μ L	X μ L	10 μ M reverse primer	0.5 μ M ^[1]	X μ L	X μ L	Template DNA	0.1–10 ng plasmid DNA (5–100 ng genomic DNA)	X μ L	X μ L				
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Run PCR

Steps	Action	Procedure details																																																	
4 	Incubate reactions in a thermal cycler	<p>3-step protocol</p> <table border="1"> <thead> <tr> <th>Cycle step</th> <th>Temperature</th> <th>Time</th> <th>Cycles</th> </tr> </thead> <tbody> <tr> <td>Initial denaturation</td> <td>98°C</td> <td>30 seconds</td> <td>1</td> </tr> <tr> <td>Denaturation</td> <td>98°C</td> <td>5–10 seconds</td> <td rowspan="3">25–35</td> </tr> <tr> <td>Annealing</td> <td>60°C</td> <td>10 seconds</td> </tr> <tr> <td>Extension</td> <td>72°C</td> <td>15–30 seconds per 1 kb</td> </tr> <tr> <td>Final extension</td> <td>72°C</td> <td>5 minutes</td> <td>1</td> </tr> <tr> <td></td> <td>4°C</td> <td>Hold</td> <td>—</td> </tr> </tbody> </table> <p>2-step protocol (for primers >30 nt long)^[1]</p> <table border="1"> <thead> <tr> <th>Cycle step</th> <th>Temperature</th> <th>Time</th> <th>Cycles</th> </tr> </thead> <tbody> <tr> <td>Initial denaturation</td> <td>98°C</td> <td>30 seconds</td> <td>1</td> </tr> <tr> <td>Denaturation</td> <td>98°C</td> <td>5–10 seconds</td> <td rowspan="2">25–35</td> </tr> <tr> <td>Annealing/Extension</td> <td>72°C</td> <td>15–30 seconds per 1 kb</td> </tr> <tr> <td>Final extension</td> <td>72°C</td> <td>5 minutes</td> <td>1</td> </tr> <tr> <td></td> <td>4°C</td> <td>hold</td> <td>—</td> </tr> </tbody> </table> <p>^[1] Without non-complementary parts (e.g. restriction tags).</p> <p>Note: Refer to “Optimization strategies”, page 1, for guidelines to optimize cycling conditions.</p>	Cycle step	Temperature	Time	Cycles	Initial denaturation	98°C	30 seconds	1	Denaturation	98°C	5–10 seconds	25–35	Annealing	60°C	10 seconds	Extension	72°C	15–30 seconds per 1 kb	Final extension	72°C	5 minutes	1		4°C	Hold	—	Cycle step	Temperature	Time	Cycles	Initial denaturation	98°C	30 seconds	1	Denaturation	98°C	5–10 seconds	25–35	Annealing/Extension	72°C	15–30 seconds per 1 kb	Final extension	72°C	5 minutes	1		4°C	hold	—
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5 	Add gel loading buffer and analyze with gel electrophoresis	<p>Add gel loading buffer to 10 µL of PCR product, mix, and briefly centrifuge the contents.</p> <p>Note: For optimal separation using E-Gel™ agarose gels, dilute the sample 2- to 20-fold. Analyze the sample using agarose gel electrophoresis.</p> <p>Use your PCR product immediately in down-stream applications, or store it at –20°C.</p>																																																	