

	Package contents	<table><tr><th>Catalog number</th><th>Size</th></tr><tr><td>12351-010</td><td>100 Units</td></tr><tr><td>12351-050</td><td>500 Units</td></tr><tr><td>12351-250</td><td>5 × 500 Units</td></tr></table>	Catalog number	Size	12351-010	100 Units	12351-050	500 Units	12351-250	5 × 500 Units	 Kit contents
Catalog number	Size										
12351-010	100 Units										
12351-050	500 Units										
12351-250	5 × 500 Units										
	Storage conditions	- Store all contents at –20°C. - Template: gDNA, plasmid DNA, phage DNA, cDNA - Forward and reverse gene-specific primers - Invitrogen™ 10 mM dNTP Mix (Cat. No. 18427-088) - 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 - Water, nuclease-free									
	Required materials										
	Timing	Varies depending on amplicon length.									
	Selection guide	PCR Enzymes and Master Mixes Go online to view related products.									
	Product description	- 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™ DNA Polymerase 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:	Separate components

PCR setup

Component	25-µL rxn	50-µL rxn	Custom	Final conc.
Water, nuclease-free	to 25 µL	to 50 µL	to µL	—
5X SuperFi™ Buffer ¹	5 µL	10 µL	µL	1X
10 mM dNTP mix	0.5 µL	1 µL	µL	0.2 mM each
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
Platinum™ SuperFi™ DNA Polymerase (2 U/µL)	0.25 µL	0.5 µL	µL	0.02 U/µL

¹ Includes 7.5 mM MgCl₂.
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

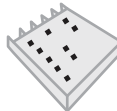
-  Click here for guidelines to optimize your PCR experiment.
-  Click here for guidelines to troubleshoot your PCR experiment.

Purchaser notification

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

The example PCR procedure below shows appropriate volumes for a single **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	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 border="1"> <thead> <tr> <th>Component</th><th>50-μL rxn</th><th>Final conc.</th></tr> </thead> <tbody> <tr> <td>Water, nuclease-free</td><td>to 50 μL</td><td></td></tr> <tr> <td>5X SuperFi™ Buffer¹</td><td>10 μL</td><td>1X</td></tr> <tr> <td>10 mM dNTP mix</td><td>1 μL</td><td>0.2 mM each</td></tr> <tr> <td>5X SuperFi™ GC Enhancer (<i>optional</i>)²</td><td>10 μL</td><td>1X</td></tr> <tr> <td>Platinum™ SuperFi™ DNA Polymerase</td><td>0.5 μL</td><td>0.02 U/μL</td></tr> </tbody> </table> ¹ Includes 7.5 mM MgCl₂. ² Recommended for targets with >65% GC sequences. Mix and then briefly centrifuge the components.	Component	50- μ L rxn	Final conc.	Water, nuclease-free	to 50 μ L		5X SuperFi™ Buffer ¹	10 μ L	1X	10 mM dNTP mix	1 μ L	0.2 mM each	5X SuperFi™ GC Enhancer (<i>optional</i>) ²	10 μ L	1X	Platinum™ SuperFi™ DNA Polymerase	0.5 μ L	0.02 U/ μ L
Component	50- μ L rxn	Final conc.																		
Water, nuclease-free	to 50 μ L																			
5X SuperFi™ Buffer ¹	10 μ L	1X																		
10 mM dNTP mix	1 μ L	0.2 mM each																		
5X SuperFi™ GC Enhancer (<i>optional</i>) ²	10 μ L	1X																		
Platinum™ SuperFi™ DNA Polymerase	0.5 μ L	0.02 U/ μ L																		
3 	Add template DNA and primers	Add your template DNA and primers to each tube for a final reaction volume of 50 μL. <table border="1"> <thead> <tr> <th>Component</th><th>50-μL rxn</th><th>Final conc.</th></tr> </thead> <tbody> <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 DNA¹</td><td>varies</td><td>varies</td></tr> </tbody> </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.	Component	50- μ L rxn	Final conc.	10 μ M forward primer	2.5 μ L	0.5 μ M	10 μ M reverse primer	2.5 μ L	0.5 μ M	Template DNA ¹	varies	varies						
Component	50- μ L rxn	Final conc.																		
10 μ M forward primer	2.5 μ L	0.5 μ M																		
10 μ M reverse primer	2.5 μ L	0.5 μ M																		
Template DNA ¹	varies	varies																		

Steps	Action	Procedure details							
4 	Incubate reactions in a thermal cycler	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 the T _m calculator on our website at www.thermofisher.com/tmcalculator to calculate the T _m of your primers and the recommended annealing temperature.									
Note: Refer to “ Optimization strategies ”, page 1, for guidelines to optimize cycling conditions.									
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.							

Package contents

Catalog number	Size
12351-010	100 Units
12351-050	500 Units
12351-250	

Storage conditions

Store all contents at -20°C.

Template: gDNA

Forward and reverse primers

Invitrogen™ Platinum™ SuperFi™ DNA Polymerase

Invitrogen™ Platinum™ SuperFi™ GC Enhancer

5X SuperFi™ Buffer

5X SuperFi™ GC Enhancer

0.2 or 0.5-mL PCR tubes

Gel loading buffer

Required materials

Enzyme characteristics

Hot-start: Antibody

Kit contents

Kit contents

CLOSE >>

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

Component	Kit sizes		
	100 Units	500 Units	2500 Units
Platinum™ SuperFi™ DNA Polymerase (2 U/µL)	50 µL	250 µL	5 × 250 µL
5X SuperFi™ Buffer	2 × 1.25 mL	6 × 1.25 mL	30 × 1.25 mL
5X SuperFi™ GC Enhancer	1.25 mL	4 × 1.25 mL	20 × 1.25 mL

Important guidelines

CLOSE >>

- 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.
- Platinum™ SuperFi™ DNA Polymerase produces blunt end DNA products.
- 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™ DNA Polymerase, it is not necessary to perform the PCR set up on ice.
- Pipet the Platinum™ SuperFi™ DNA Polymerase carefully and gently. Otherwise, the high glycerol content (50%) in the storage buffer may lead to pipetting errors.
- 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 DNA Polymerase (Cat. No. 12357-010) with 5X SuperFi™ Green Buffer, which contains tracking dyes and a density reagent for direct loading of PCR products on gels.

separate vial of SuperFi™ GC Enhancer designed for GC-rich templates (>65% GC).

Click here for guidelines to troubleshoot your PCR experiment.

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.

Purchaser notification

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

Optimization strategies

CLOSE >>

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).

Mg²⁺: SuperFi™ Buffer provides MgCl₂ 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 Mg²⁺ optimum may be shifted to higher concentrations.
- If further optimization is needed, increase the Mg²⁺ 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.

Troubleshooting

CLOSE >>

Observation	Recommended action
No product at all or low yield.	▪ Make sure that there are no pipetting errors. ▪ Use fresh high-quality dNTPs. Do not use dNTP mix or primers 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

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

CLOSE >>

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

Disclaimer

TO THE EXTENT ALLOWED BY LAW, LIFE TECHNOLOGIES AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

Important licensing information

These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

Corporate Entity: Life Technologies | Carlsbad, CA 92008 USA | Toll free in USA 1.800.955.6288

© 2017 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific and its subsidiaries.

Steps

4

5

10kb)

Time

2 min

10 sec

10 sec

10 sec/kb

5 min

hold

late the T_m of