

	Package contents	Catalog number	Size	 Kit contents
		12358-010	100 rxns	
		12358-050	500 rxns	
		12358-250	5 × 500 rxns	

	Storage conditions	▪ Store all contents at –20°C.
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	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
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	Timing	Varies depending on amplicon length
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	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.
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	Online resources	Visit our product page for additional information and protocols. For support, visit thermofisher.com/support .
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Enzyme characteristics

Hot-start:	Antibody
Length:	Up to 20 kb
Fidelity vs. <i>Taq</i>:	>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 MgCl₂ 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.

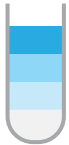
Optimization strategies and troubleshooting

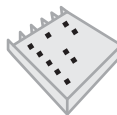
-  Click here for guidelines to optimize your PCR experiment.
-  Click here for guidelines to troubleshoot your PCR experiment.

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.

Steps	Action	Procedure details												
1 	Thaw reagents	Thaw, mix, and briefly centrifuge each component before use. Avoid generating bubbles when mixing the Master Mix.												
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>2X Platinum™ SuperFi™ PCR Master Mix</td><td>25 μL</td><td>1X</td></tr> <tr> <td>5X SuperFi™ GC Enhancer (<i>optional</i>)¹</td><td>10 μL</td><td>1X</td></tr> </tbody> </table> ¹ Recommended for targets with >65% GC sequences. Mix and then briefly centrifuge the components.	Component	50-μL rxn	Final conc.	Water, nuclease-free	to 50 μL		2X Platinum™ SuperFi™ PCR Master Mix	25 μL	1X	5X SuperFi™ GC Enhancer (<i>optional</i>) ¹	10 μL	1X
Component	50-μL rxn	Final conc.												
Water, nuclease-free	to 50 μL													
2X Platinum™ SuperFi™ PCR Master Mix	25 μL	1X												
5X SuperFi™ GC Enhancer (<i>optional</i>) ¹	10 μL	1X												
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-μL rxn	Final conc.	10 μM forward primer	2.5 μL	0.5 μM	10 μM reverse primer	2.5 μL	0.5 μM	Template DNA ¹	varies	varies
Component	50-μL rxn	Final conc.												
10 μM forward primer	2.5 μL	0.5 μM												
10 μM reverse primer	2.5 μL	0.5 μM												
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 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.									
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.							