Invitrogen[™] Platinum[™] SuperFi[™] II Green PCR Master Mix

invitrogen

USER GUIDE			Pub. No. MAN0018	861	Rev. A.0		
		Catalog number	Size				
S	Package contents	12369010	100 reactions				
		12369050	500 reactions	W Kit o	contents		
		12369250	5×500 reactions				
	Storage conditions	 Store all content 	ts at −20°C.				

Required materials

🚺 Click here for required materials

- 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.
- Platinum[™] SuperFi[™] II Green PCR Master Mix is a readyto-use mixture of DNA polymerase, salts, magnesium, and dNTPs for efficient PCR amplification, which retains all the features of the Platinum[™] SuperFi[™] II DNA Polymerase. In addition, it is supplemented with two tracking dyes and a density reagent for direct loading of PCR products on gels.
- 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

Product description

- 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.

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PCR Enzymes and Master Mixes Go online to view related products.

Online 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:	>300X
Timing:	Varies depending on amplicon length
Format:	Master Mix

PCR setup

Component	Final concentration	20-µL rxn	50-µL rxn	
2X Platinum [™] SuperFi [™] II Green PCR Master Mix ^[1]	1X	10 µL	25 µL	
Forward primer	$0.5 \mu M^{[2]}$	XμL	XμL	
Reverse primer	0.5 μM ^[2]	XμL	XμL	
Template DNA	0.1–10 ng plasmid (5–100 ng genomic DNA)	XμL	XμL	
Water, nuclease-free		— to 20 μL to		

^[1] Provides 1.5 mM MgCl₂ in 1X concentration.

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

Important guidelines

Olick 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

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Prepare and run PCR

The example PCR procedure below shows appropriate volumes for a single **20-µL** or **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-mL or 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.							
	&	Prepare reaction mix with template DNA and primers	Add the following components to each PCR tube. Note: Consider the volumes for all components in reaction mix to determine the correct amount of water required to reach your final reaction volume.							
			Component			Final concentration		20-µL rxn	50-µL rxn	
			2X Platinum [™] SuperFi [™] II Green PCR Master Mix ^[1]		Mix ^[1]	1X		10 µL	25 µL	
			Forward primer			0.5 µM ^[2]		XμL	XμL	
2	K		Reverse primer			0.5 µM ^[2]		XμL	XμL	
2			Template DNA			0.1–10 ng plasmid (5–100 ng genomic DNA)		XμL	XμL	
			Water, nuclease-free			_		to 20 µL	to 50 μL	
			 ^[1] Provides 1.5 mM MgCl₂ in ^[2] Reduce the primer concent reactions. Cap each tube, mix, and the 	1X concentration. ration to 0.2 μM final f en briefly centrifuge	for amplifice e the cont	cation of >5 kb targ ents.	ets from g	enomic DNA and	l for multiplex	
			3-step protocol							
			Cycle step	Temperature		Time	Cycles			
			Initial denaturation	98°C	3	0 seconds	1			
		Incubate reactions in a	Denaturation	98°C	5-1	10 seconds				
3		thermal cycler (3-step protocol)	Annealing	60°C	1	10 seconds 25–35				
			Extension	72°C	15–30 s	econds per 1 kb				
			Final extension	72°C 4°C	5	minutes Hold	1			
			Note: Refer to "Optimiz	ation strategies", pa	age 1, for	guidelines to opti	mize cyc	ling conditions.		

	Steps	Action	Procedure details					
3		Incubate reactions in a thermal cycler (2-step protocol)	2-step protocol (for primers >30 nt long) ^[1]					
			Cycle step	Temperature	Time			
			Initial denaturation	98°C	30 seconds	1		
			Denaturation	98°C	5–10 seconds	25.25		
			Annealing/Extension	72°C	15–30 seconds per 1 kb	23-33		
			Final extension	72°C	5 minutes	1		
				4°C	hold	—		
			^[1] Without non-complementary parts (e.g. restriction tags).					
			Note: Refer to "Optimization strategies", page 1, for guidelines to optimize cycling conditions.					
	Harris Constraints	Add gel loading buffer and analyze with gel electrophoresis	Analyze the sample using agarose gel electrophoresis.					
4			Note: For optimal separation using E-Gel [™] agarose gels, dilute the sample 2- to 20-fold.					
			Note: PCR mixes prepared using the Platinum [™] SuperFi [™] II Green PCR Master Mix 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.					

