

	Catalog No.	Size	
Package contents	14966-001	100 reactions	Kit contents
	14966-005	500 reactions	
	14966-025	2500 reactions	

Storage conditions	
	Store all contents at -20°C .

Required materials	
	<ul style="list-style-type: none"> Template: cDNA, genomic DNA, plasmid DNA, phage DNA Forward and reverse primers Invitrogen™ 10 mM dNTP mix (Cat. no. 18427-088) Water, nuclease-free Invitrogen™ E-Gel™ EX Agarose Gels, 1% (Cat. No. G4010-01) Invitrogen™ E-Gel™ 1 kb Plus Express DNA Ladder (Cat. No. 10488-091) 0.2 or 0.5-mL nuclease-free microcentrifuge tubes Gel loading buffer

Timing	
	Varies depending on amplicon length.

	<ul style="list-style-type: none"> Invitrogen™ Platinum™ II Taq Hot-Start DNA Polymerase is an engineered Taq DNA polymerase that shows increased resistance to reaction inhibitors originating from sample material or DNA purification steps. The polymerase activity is blocked at ambient temperatures and restored after the initial denaturation step at 94°C. This automatic “hot start” provides increased sensitivity, specificity, and yield, while allowing reaction assembly at room temperature. Due to unique composition of the Platinum™ II PCR buffer, the annealing temperature is 60°C for most primer pairs designed following general primer design rules. Platinum™ II Taq DNA polymerase extends 1 kb in 15 seconds. The extension step can be prolonged without a negative effect on specificity. The enzyme has a template independent terminal transferase activity that adds a single deoxyadenosine (A) to the 3' ends of PCR products. Like standard Taq, it has both 5' to 3' polymerase and 5' to 3' exonuclease activities, but lacks 3' to 5' exonuclease activity.
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Online resources	
	Visit our product page for additional information. Find out more at thermofisher.com/platinumiiataq . For support, visit thermofisher.com/support .

Enzyme characteristics

Hot-start:	Antibody
Length:	Up to 5 kb
Fidelity vs. Taq:	1X
Format:	Separate components

PCR setup

Use the measurements below to prepare your PCR experiment, or enter your own parameters in the column provided.

Component	20- μL rxn	50- μL rxn	Custom	Final conc.
Water, nuclease-free	to 20 μL	to 50 μL	to μL	—
5X Platinum™ II PCR Buffer ¹	4 μL	10 μL	μL	1X
10 mM dNTP mix	0.4 μL	1 μL	μL	0.2 mM each
10 μM forward primer	0.4 μL	1 μL	μL	0.2 μM
10 μM reverse primer	0.4 μL	1 μL	μL	0.2 μM
Template DNA ²	varies	varies	μL	<500 ng/rxn
Platinum™ GC Enhancer (optional) ³	4 μL	10 μL	μL	1X
Platinum™ II Taq Hot-Start DNA Polymerase	0.16 μL	0.4 μL	μL	0.04 U/ μL

¹ Provides 1.5 mM MgCl_2 in final reaction concentration.

² 0.5–500 ng genomic DNA, 1 pg–50 ng plasmid or viral DNA, or 1–5 μL of cDNA synthesis reaction per 50- μL PCR reaction.

³ Recommended for targets with >65% GC sequences.

PCR protocol

Go to page 2 for instructions to prepare and run your PCR experiment.

Important guidelines

Click here for important PCR guidelines.

Optimization strategies

Click here for guidelines to optimize your PCR experiment.




Troubleshooting



Click here to troubleshoot your PCR experiment.

Limited warranty, disclaimer, and licensing information

PCR protocol

The example procedure below shows the 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 the 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.																		
2 	Prepare PCR master mix	<p>a. Add the following components to each reaction 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>Volume for 50-μL rxn</th> <th>Final concentration</th> </tr> </thead> <tbody> <tr> <td>Water, nuclease-free</td> <td>to 50 μL</td> <td>—</td> </tr> <tr> <td>5X Platinum™ II PCR Buffer</td> <td>10 μL</td> <td>1X</td> </tr> <tr> <td>10 mM dNTP mix (10 mM each)</td> <td>1 μL</td> <td>0.2 mM each</td> </tr> <tr> <td>Platinum™ GC Enhancer (<i>optional</i>)¹</td> <td>10 μL</td> <td>1X</td> </tr> <tr> <td>Platinum™ II <i>Taq</i> Hot-Start DNA Polymerase</td> <td>0.4 μL</td> <td>0.04 U/μL</td> </tr> </tbody> </table> <p>¹ Recommended for targets with >65% GC sequences.</p> <p>b. Mix, then briefly centrifuge the components.</p>	Component	Volume for 50- μ L rxn	Final concentration	Water, nuclease-free	to 50 μ L	—	5X Platinum™ II PCR Buffer	10 μ L	1X	10 mM dNTP mix (10 mM each)	1 μ L	0.2 mM each	Platinum™ GC Enhancer (<i>optional</i>) ¹	10 μ L	1X	Platinum™ II <i>Taq</i> Hot-Start DNA Polymerase	0.4 μ L	0.04 U/ μ L
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3 	Add template DNA and primers	<p>a. Add your template DNA and primers to each tube for a final reaction volume of 50 μL.</p> <table border="1"> <thead> <tr> <th>Component</th> <th>Volume for 50-μL rxn</th> <th>Final concentration</th> </tr> </thead> <tbody> <tr> <td>10 μM forward primer</td> <td>1 μL</td> <td>0.2 μM</td> </tr> <tr> <td>10 μM reverse primer</td> <td>1 μL</td> <td>0.2 μM</td> </tr> <tr> <td>Template DNA</td> <td>varies</td> <td><500 ng/rxn</td> </tr> </tbody> </table> <p>b. Cap each tube, mix, then briefly centrifuge the contents.</p>	Component	Volume for 50- μ L rxn	Final concentration	10 μ M forward primer	1 μ L	0.2 μ M	10 μ M reverse primer	1 μ L	0.2 μ M	Template DNA	varies	<500 ng/rxn						
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<p>4 </p>	<p>Incubate reactions in a thermal cycler</p>	<table border="1" data-bbox="695 126 1780 435"> <thead> <tr> <th rowspan="2">Step</th> <th colspan="2">3-step protocol</th> <th colspan="2">2-step protocol¹</th> </tr> <tr> <th>Temperature</th> <th>Time</th> <th>Temperature</th> <th>Time</th> </tr> </thead> <tbody> <tr> <td>Initial denaturation</td> <td>94°C</td> <td>2 minutes</td> <td>94°C</td> <td>2 minutes</td> </tr> <tr> <td rowspan="3">25–35 PCR cycles</td> <td>Denature</td> <td>94°C</td> <td>98°C</td> <td>5 seconds</td> </tr> <tr> <td>Anneal²</td> <td>60°C</td> <td rowspan="2">60°C</td> <td rowspan="2">15 seconds</td> </tr> <tr> <td>Extend</td> <td>68°C</td> <td>15 seconds/kb</td> </tr> <tr> <td>Hold</td> <td>4°C</td> <td>hold</td> <td>4°C</td> <td>hold</td> </tr> </tbody> </table> <p>¹ Recommended for simple amplicons up to 1 kb with 45–65% GC sequences. For longer, GC-rich, and complex amplicons, or cDNA targets, use the 3-step cycling protocol.</p> <p>² 60°C annealing temperature works for most primers. In cases when annealing temperature requires additional optimization, we recommend performing gradient PCR or redesigning the primers.</p> <p>Note: Refer to “Optimization strategies”, page 1, for guidelines to optimize cycling conditions.</p>	Step	3-step protocol		2-step protocol ¹		Temperature	Time	Temperature	Time	Initial denaturation	94°C	2 minutes	94°C	2 minutes	25–35 PCR cycles	Denature	94°C	98°C	5 seconds	Anneal ²	60°C	60°C	15 seconds	Extend	68°C	15 seconds/kb	Hold	4°C	hold	4°C	hold
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<p>5 </p>	<p>Add gel loading buffer and analyze with gel electrophoresis</p>	<p>a. Add gel loading buffer to 10 µL of PCR sample, mix, and briefly centrifuge the contents. Note: Dilute the PCR sample 2- to 20-fold for optimal separation on E-Gel™ agarose gels.</p> <p>b. Analyze the sample using agarose gel electrophoresis.</p> <p>c. Use your PCR product immediately in down-stream applications, or store it at –20°C.</p>																															