

	Package contents	Catalog number EP147B002	Size 2500 rxns	Kit contents
	Storage conditions	<ul style="list-style-type: none"> Store all contents at -20°C. Template: cDNA, gDNA, λDNA Forward and reverse gene-specific primers Invitrogen™ 10 mM dNTP mix (Cat. no. 18427-088) Water, nuclease-free 0.2 or 0.5-mL nuclease-free microcentrifuge tubes for qPCR: <ul style="list-style-type: none"> 100 μM primer probe Invitrogen™ ROX Reference Dye (Cat. no. 12223-012) for PCR: <ul style="list-style-type: none"> Invitrogen™ E-Ge™ General Purpose Gels, 1.2% (Cat. no. G5018-01) Invitrogen™ TrackIt™ 1 kb Plus DNA Ladder (Cat. no. 10488-085) 		
	Required materials	<ul style="list-style-type: none"> for qPCR: <ul style="list-style-type: none"> 100 μM primer probe Invitrogen™ ROX Reference Dye (Cat. no. 12223-012) for PCR: <ul style="list-style-type: none"> Invitrogen™ E-Ge™ General Purpose Gels, 1.2% (Cat. no. G5018-01) Invitrogen™ TrackIt™ 1 kb Plus DNA Ladder (Cat. no. 10488-085) 		
	Timing	<p>Varies depending on amplicon length</p> <ul style="list-style-type: none"> Low Glycerol Platinum™ Taq, CG DNA Polymerase is a recombinant Taq polymerase complexed with a proprietary antibody that blocks polymerase activity at ambient temperatures. Activity is restored after the initial denaturation step in PCR cycling at 95°C, providing an automatic “hot start” and offering increased sensitivity, specificity, and yield, while allowing reaction assembly at room temperature. This enzyme has a non-template-dependent, 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 activity, but lacks 3' to 5' exonuclease activity. Low Glycerol Platinum™ Taq, CG DNA Polymerase is supplied at a concentration of 50 U/μL with minimal glycerol content, rendering it feasible for lyophilization applications. 		
	Important guidelines	Click here for important PCR guidelines.		
	Online resources	For further information, contact outlicensing@lifetech.com .		

Enzyme characteristics

Hot-start:	Antibody
Length:	Up to 4 kb
Fidelity vs. Taq:	1X
Format:	Separate components

qPCR setup

Use the measurements below to prepare your qPCR experiment, or enter your own parameters in the column provided. For PCR set-up, see page 2.

Component	50- μL rxn	Custom	Final conc. in 50- μL rxn
Water, nuclease-free	to 50 μL	to μL	—
10X PCR Buffer (– MgCl_2)	5 μL	μL	1X
50 mM MgCl_2	1.5 μL	μL	1.5 mM
10 mM dNTP mix	1 μL	μL	0.2 mM each
10 μM forward primer	1.5 μL	μL	0.3 μM
10 μM reverse primer	1.5 μL	μL	0.3 μM
100 μM primer probe	0.1 μL	μL	0.2 μM
30 μM ROX Reference Dye	0.05 μL	μL	30 nM
Template DNA	varies	μL	≤ 500 ng/rxn
Low Glycerol Platinum™ Taq, CG DNA Polymerase (10 U/ μL) ¹	0.2 μL	μL	2 U/rxn

¹ Prior to use, dilute the Low Glycerol Platinum™ Taq, CG DNA Polymerase to at least 10 U/ μL with Low Glycerol Platinum™ Taq, CG Diluent included in the kit.

PCR protocol

See pages 2 and 3 for instructions to prepare and run your PCR experiment.


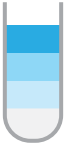

Optimization strategies


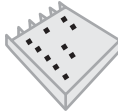
Click here for guidelines to optimize your PCR experiment.

Purchaser notification

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The example 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, and then dispense appropriate volumes into each 0.2–0.5 mL PCR tube or well of a MicroAmp™ EnduraPlate™ Optical 96- or 384-well plate prior to adding template DNA and primers. For 384-well plates, we recommend a maximum reaction volume of 10 μ L per well.

Step	Action	Procedure details																																								
1 	Thaw reagents	<p>Thaw, mix, and briefly centrifuge each component before use.</p> <p>Prior to use, dilute the Low Glycerol Platinum™ Taq, CG DNA Polymerase (50 U/μL) to at least 10 U/μL in Low Glycerol Platinum™ Taq, CG Diluent included in the kit.</p> <p>Note: After dilution, the Low Glycerol Platinum™ Taq, CG DNA Polymerase (10 U/μL) can be stored at 4°C for use within 1–2 days. Do not store the diluted enzyme at –20°C.</p>																																								
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>50-μL rxn</th> <th>Custom</th> <th>Final conc.</th> </tr> </thead> <tbody> <tr> <td>Water, nuclease-free</td> <td>to 50 μL</td> <td>μL</td> <td>—</td> </tr> <tr> <td>10X PCR Buffer (– MgCl₂)</td> <td>5 μL</td> <td>μL</td> <td>1X</td> </tr> <tr> <td>50 mM MgCl₂</td> <td>1.5 μL</td> <td>μL</td> <td>1.5 mM</td> </tr> <tr> <td>10 mM dNTP mix</td> <td>1 μL</td> <td>μL</td> <td>0.2 mM each</td> </tr> <tr> <td>Low Glycerol Platinum™ Taq, CG DNA Polymerase (10 U/μL)</td> <td>0.2 μL</td> <td>μL</td> <td>2 U/rxn</td> </tr> </tbody> </table> <p>Mix and then briefly centrifuge the components.</p>	Component	50- μ L rxn	Custom	Final conc.	Water, nuclease-free	to 50 μ L	μ L	—	10X PCR Buffer (– MgCl ₂)	5 μ L	μ L	1X	50 mM MgCl ₂	1.5 μ L	μ L	1.5 mM	10 mM dNTP mix	1 μ L	μ L	0.2 mM each	Low Glycerol Platinum™ Taq, CG DNA Polymerase (10 U/ μ L)	0.2 μ L	μ L	2 U/rxn																
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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 50 μL.</p> <p>For qPCR:</p> <table border="1"> <thead> <tr> <th>Component</th> <th>50-μL rxn</th> <th>Custom</th> <th>Final conc.</th> </tr> </thead> <tbody> <tr> <td>10 μM forward gene-specific primer</td> <td>1.5 μL</td> <td>μL</td> <td>0.3 μM</td> </tr> <tr> <td>10 μM reverse gene-specific primer</td> <td>1.5 μL</td> <td>μL</td> <td>0.3 μM</td> </tr> <tr> <td>100 μM primer probe</td> <td>0.1 μL</td> <td>μL</td> <td>0.2 μM</td> </tr> <tr> <td>30 μM ROX Reference Dye¹</td> <td>0.05 μL</td> <td>μL</td> <td>30 nM</td> </tr> <tr> <td>Template DNA</td> <td>varies</td> <td>μL</td> <td>\leq500 ng/rxn (human gDNA)¹</td> </tr> </tbody> </table> <p>¹ See “Optimization strategies”, page 1.</p> <p>For PCR:</p> <table border="1"> <thead> <tr> <th>Component</th> <th>50-μL rxn</th> <th>Custom</th> <th>Final conc.</th> </tr> </thead> <tbody> <tr> <td>10 μM forward gene-specific primer</td> <td>1 μL</td> <td>μL</td> <td>0.2 μM</td> </tr> <tr> <td>10 μM reverse gene-specific primer</td> <td>1 μL</td> <td>μL</td> <td>0.2 μM</td> </tr> <tr> <td>Template DNA</td> <td>varies</td> <td>μL</td> <td>\leq500 ng/rxn (human gDNA)¹</td> </tr> </tbody> </table> <p>¹ See “Optimization strategies”, page 1.</p> <p>Cap each tube, mix, and then briefly centrifuge the contents.</p>	Component	50- μ L rxn	Custom	Final conc.	10 μ M forward gene-specific primer	1.5 μ L	μ L	0.3 μ M	10 μ M reverse gene-specific primer	1.5 μ L	μ L	0.3 μ M	100 μ M primer probe	0.1 μ L	μ L	0.2 μ M	30 μ M ROX Reference Dye ¹	0.05 μ L	μ L	30 nM	Template DNA	varies	μ L	\leq 500 ng/rxn (human gDNA) ¹	Component	50- μ L rxn	Custom	Final conc.	10 μ M forward gene-specific primer	1 μ L	μ L	0.2 μ M	10 μ M reverse gene-specific primer	1 μ L	μ L	0.2 μ M	Template DNA	varies	μ L	\leq 500 ng/rxn (human gDNA) ¹
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5	 Analyze results	<ul style="list-style-type: none"> Analyze results following your real-time instrument manufacturer’s guidelines. You can check the specificity of the PCR/qPCR products by agarose gel electrophoresis. Before loading, add gel loading buffer to 10 µL of the PCR/qPCR sample, mix, and briefly centrifuge the contents. You can store your samples overnight at 2–8°C, or at –20°C for longer storage. 																																																							

References

- SantaLucia, Jr, J. (1998) A unified view of polymer, dumbbell and oligonucleotide DNA nearest-neighbor thermodynamics. *Proc. Natl Acad. Sci.*, 95, 1460–1465.
 SantaLucia, Jr, J. and Hicks, D. (2004) The thermodynamics of DNA structural motifs. *Annu. Rev. Biophys. Biomol. Struct.*, 33, 415–440.