

	<b>Package contents</b>	Catalog number EP1530DFSMP	Size 800 rxns	Kit contents
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	<b>Storage conditions</b>	<ul style="list-style-type: none"> <li>Store all contents at <math>-20^{\circ}\text{C}</math>.</li> </ul>
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	<b>Required materials</b>	<ul style="list-style-type: none"> <li>Template: cDNA, gDNA</li> <li>Forward and reverse gene-specific primers</li> <li>Invitrogen™ 10 mM dNTP mix (Cat. No. 18427-088)</li> <li>Water, nuclease-free</li> <li>0.2-mL or 0.5-mL nuclease-free microcentrifuge tubes</li> <li>for qPCR:                             <ul style="list-style-type: none"> <li>100 <math>\mu\text{M}</math> qPCR probe</li> <li>ROX Reference Dye</li> </ul> </li> <li>for PCR:                             <ul style="list-style-type: none"> <li>Invitrogen™ E-Ge™ General Purpose Gels, 1.2% (Cat. No. G5018-01)</li> <li>Invitrogen™ TrackIt™ 1 kb Plus DNA Ladder (Cat. No. 10488-085)</li> </ul> </li> </ul>
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	<b>Timing</b>	<p>Varies depending on amplicon length</p> <ul style="list-style-type: none"> <li>Invitrogen™ Platinum™ Taq DNA Polymerase, DNA-free is manufactured using closed and single-use system technology to minimize DNA contamination risk.</li> <li>Platinum™ Taq DNA Polymerase, DNA-free is a recombinant Taq polymerase complexed with a proprietary antibody that blocks polymerase activity at ambient temperatures.</li> <li>Activity is restored after the initial denaturation step in PCR cycling at <math>94^{\circ}\text{C}</math>, providing an automatic “hot start” and offering increased sensitivity, specificity, and yield, while allowing reaction assembly at room temperature.</li> <li>This enzyme has a non-template-dependent, terminal transferase activity that adds a single deoxyadenosine (A) to the 3' ends of PCR products.</li> <li>Like standard Taq, it has both 5' to 3' polymerase and 5' to 3' exonuclease activities, but lacks 3' to 5' exonuclease activity.</li> </ul>
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	<b>Important guidelines</b>	Click here for important PCR guidelines.
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	<b>Online resources</b>	Visit our <a href="http://thermofisher.com/dna-free">thermofisher.com/dna-free</a> for additional information.
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For Research Use Only. Not for use in diagnostic procedures.

## Enzyme characteristics

<b>Hot-start:</b>	Antibody
<b>Length:</b>	Up to 4 kb
<b>Fidelity vs. Taq:</b>	1X
<b>Format:</b>	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	25- $\mu\text{L}$ rxn	Custom	Final conc. in 25- $\mu\text{L}$ rxn
Water, nuclease-free	to 25 $\mu\text{L}$	to $\mu\text{L}$	—
10X PCR Buffer (– $\text{MgCl}_2$ ), DNA-free	2.5 $\mu\text{L}$	$\mu\text{L}$	1X
50 mM $\text{MgCl}_2$ , DNA-free	0.75 $\mu\text{L}$	$\mu\text{L}$	1.5 mM
10 mM dNTP mix	0.5 $\mu\text{L}$	$\mu\text{L}$	0.2 mM each
10 $\mu\text{M}$ forward primer	0.75 $\mu\text{L}$	$\mu\text{L}$	0.3 $\mu\text{M}$
10 $\mu\text{M}$ reverse primer	0.75 $\mu\text{L}$	$\mu\text{L}$	0.3 $\mu\text{M}$
100 $\mu\text{M}$ qPCR probe	0.05 $\mu\text{L}$	$\mu\text{L}$	0.2 $\mu\text{M}$
30 $\mu\text{M}$ ROX Reference Dye	0.025 $\mu\text{L}$	$\mu\text{L}$	30 nM <sup>1</sup>
Template DNA	varies	$\mu\text{L}$	$\leq 500$ ng/rxn
Platinum™ Taq DNA Polymerase, DNA-free (5 U/ $\mu\text{L}$ )	0.25 $\mu\text{L}$	$\mu\text{L}$	1.25 U/rxn

<sup>1</sup>The recommended final ROX concentration depends on the instrument (see “Important guidelines”).

## PCR protocol

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


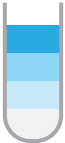

## Optimization strategies


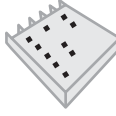
Click here for guidelines to optimize your PCR experiment.

## Purchaser notification

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The example procedure below shows appropriate volumes for a single **25- $\mu$ 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  $\mu$ L per well.

Step	Action	Procedure details																																								
1 	<b>Thaw reagents</b>	Thaw, mix, and briefly centrifuge each component before use.																																								
2 	<b>Prepare PCR master mix</b>	<p>a. Add the following components to each PCR tube.</p> <p><b>Note:</b> 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>25-<math>\mu</math>L rxn</th> <th>Custom</th> <th>Final conc.</th> </tr> </thead> <tbody> <tr> <td>Water, nuclease-free</td> <td>to 25 <math>\mu</math>L</td> <td><math>\mu</math>L</td> <td>—</td> </tr> <tr> <td>10X PCR Buffer (–MgCl<sub>2</sub>), DNA-free</td> <td>2.5 <math>\mu</math>L</td> <td><math>\mu</math>L</td> <td>1X</td> </tr> <tr> <td>50 mM MgCl<sub>2</sub>, DNA-free</td> <td>0.75 <math>\mu</math>L</td> <td><math>\mu</math>L</td> <td>1.5 mM</td> </tr> <tr> <td>10 mM dNTP mix</td> <td>0.5 <math>\mu</math>L</td> <td><math>\mu</math>L</td> <td>0.2 mM each</td> </tr> <tr> <td>Platinum™ Taq DNA Polymerase, DNA-free (5 U/<math>\mu</math>L)</td> <td>0.25 <math>\mu</math>L</td> <td><math>\mu</math>L</td> <td>1.25 U/rxn</td> </tr> </tbody> </table> <p>b. Mix and then briefly centrifuge the components.</p>	Component	25- $\mu$ L rxn	Custom	Final conc.	Water, nuclease-free	to 25 $\mu$ L	$\mu$ L	—	10X PCR Buffer (–MgCl <sub>2</sub> ), DNA-free	2.5 $\mu$ L	$\mu$ L	1X	50 mM MgCl <sub>2</sub> , DNA-free	0.75 $\mu$ L	$\mu$ L	1.5 mM	10 mM dNTP mix	0.5 $\mu$ L	$\mu$ L	0.2 mM each	Platinum™ Taq DNA Polymerase, DNA-free (5 U/ $\mu$ L)	0.25 $\mu$ L	$\mu$ L	1.25 U/rxn																
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3 	<b>Add template DNA and primers</b>	<p>a. Add your template DNA and primers to each tube for a final reaction volume of 25 <math>\mu</math>L.</p> <p><b>For qPCR<sup>1</sup>:</b></p> <table border="1"> <thead> <tr> <th>Component</th> <th>25-<math>\mu</math>L rxn</th> <th>Custom</th> <th>Final conc.</th> </tr> </thead> <tbody> <tr> <td>10 <math>\mu</math>M forward gene-specific primer</td> <td>0.75 <math>\mu</math>L</td> <td><math>\mu</math>L</td> <td>0.3 <math>\mu</math>M</td> </tr> <tr> <td>10 <math>\mu</math>M reverse gene-specific primer</td> <td>0.75 <math>\mu</math>L</td> <td><math>\mu</math>L</td> <td>0.3 <math>\mu</math>M</td> </tr> <tr> <td>100 <math>\mu</math>M qPCR probe</td> <td>0.05 <math>\mu</math>L</td> <td><math>\mu</math>L</td> <td>0.2 <math>\mu</math>M</td> </tr> <tr> <td>30 <math>\mu</math>M ROX Reference Dye</td> <td>0.025 <math>\mu</math>L</td> <td><math>\mu</math>L</td> <td>30 nM<sup>2</sup></td> </tr> <tr> <td>Template DNA</td> <td>varies</td> <td><math>\mu</math>L</td> <td><math>\leq</math>500 ng/rxn (human gDNA)</td> </tr> </tbody> </table> <p><sup>1</sup> See “Optimization strategies”, page 1.  <sup>2</sup> The recommended final ROX concentration depends on the instrument (see “Important guidelines”, page 1).</p> <p><b>For PCR<sup>1</sup>:</b></p> <table border="1"> <thead> <tr> <th>Component</th> <th>25-<math>\mu</math>L rxn</th> <th>Custom</th> <th>Final conc.</th> </tr> </thead> <tbody> <tr> <td>10 <math>\mu</math>M forward gene-specific primer</td> <td>0.5 <math>\mu</math>L</td> <td><math>\mu</math>L</td> <td>0.2 <math>\mu</math>M</td> </tr> <tr> <td>10 <math>\mu</math>M reverse gene-specific primer</td> <td>0.5 <math>\mu</math>L</td> <td><math>\mu</math>L</td> <td>0.2 <math>\mu</math>M</td> </tr> <tr> <td>Template DNA</td> <td>varies</td> <td><math>\mu</math>L</td> <td><math>\leq</math>500 ng/rxn (human gDNA)</td> </tr> </tbody> </table> <p><sup>1</sup> See “Optimization strategies”, page 1.</p> <p>b. Cap each tube, mix, and then briefly centrifuge the contents.</p>	Component	25- $\mu$ L rxn	Custom	Final conc.	10 $\mu$ M forward gene-specific primer	0.75 $\mu$ L	$\mu$ L	0.3 $\mu$ M	10 $\mu$ M reverse gene-specific primer	0.75 $\mu$ L	$\mu$ L	0.3 $\mu$ M	100 $\mu$ M qPCR probe	0.05 $\mu$ L	$\mu$ L	0.2 $\mu$ M	30 $\mu$ M ROX Reference Dye	0.025 $\mu$ L	$\mu$ L	30 nM <sup>2</sup>	Template DNA	varies	$\mu$ L	$\leq$ 500 ng/rxn (human gDNA)	Component	25- $\mu$ L rxn	Custom	Final conc.	10 $\mu$ M forward gene-specific primer	0.5 $\mu$ L	$\mu$ L	0.2 $\mu$ M	10 $\mu$ M reverse gene-specific primer	0.5 $\mu$ L	$\mu$ L	0.2 $\mu$ M	Template DNA	varies	$\mu$ L	$\leq$ 500 ng/rxn (human gDNA)
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<p>5</p> 	<p><b>Analyze results</b></p>	<ol style="list-style-type: none"> <li>Analyze results following your real-time instrument manufacturer’s guidelines.</li> <li>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.</li> <li>You can store your samples overnight at 2–8°C, or at –20°C for longer period.</li> </ol>																																															