Invitrogen™ Low Glycerol LibertyTaq™ DNA Polymerase

USER GUIDE Pub. no. MAN0014568 Rev. A.0

invitrogen

Print Options

Package contents

Catalog number	Size	
EP150B005	5000 units	Kit contents

Storage conditions

- Store all contents at –20°C.

Required materials

- Template: cDNA, gDNA, λDNA
- Forward and reverse gene-specific primers
- Invitrogen™ 10 mM dNTP mix (Cat. no. 18427-088)
- 100 µM primer probe
- Water, nuclease-free
- Invitrogen™ ROX Reference Dye (Cat. no. 12223-012)
- 0.2 or 0.5-mL nuclease-free microcentrifuge tubes

Timing

Varies depending on amplicon length

Product description

- Low Glycerol LibertyTaq™ DNA Polymerase is a recombinant *Taq* polymerase complexed with a proprietary hot-start component that blocks polymerase activity at ambient temperatures.
- Activity is restored past 57°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 LibertyTaq™ DNA Polymerase is supplied at a concentration of 50 U/µL with minimal glycerol content, rendering it feasible for lyophilization applications.
- The Low Glycerol LibertyTaq™ DNA Polymerase is designed for qPCR applications.

Important guidelines

Click here for important qPCR guidelines.

Online resources

For further information, contact outlicensing@lifetech.com.

For Research Use Only. Not for use in diagnostic procedures.

Enzyme characteristics

Hot-start: Proprietary
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.

Component	25-µL rxn	Custom	Final conc. in 25-µL rxn
Water, nuclease-free	to 25 µL	to µL	—
20X Liberty PCR Buffer (– $MgCl_2$)	1.25 µL	µL	1X
50 mM $MgCl_2$	0.75 µL	µL	1.5 mM
10 mM dNTP mix	0.5 µL	µL	0.2 mM each
10 µM forward primer	0.75 µL	µL	0.3 µM
10 µM reverse primer	0.75 µL	µL	0.3 µM
100 µM primer probe	0.05 µL	µL	0.2 µM
30 µM ROX Reference Dye	0.025 µL	µL	30 nM
Template DNA	varies	µL	≤250 ng/rxn
Low Glycerol LibertyTaq™ DNA Polymerase (10 U/µL)[1]	0.1 µL	µL	1 U/rxn

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

qPCR protocol

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

Optimization strategies

Click here for guidelines to optimize your qPCR experiment.

Purchaser notification

Click here for Limited Warranty, Disclaimer, and Licensing information.

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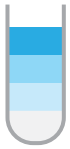
The example qPCR procedure below shows appropriate volumes for a single **25-µL** reaction. For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, and then dispense appropriate volumes into each 0.2–0.5 mL qPCR reaction tube or well of a MicroAmp™ 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 µL per well.

<table>
<tr><th>Steps</th><th>Action</th><th>Procedure details</th></tr>
<tr><td>1  </td><td>Thaw reagents</td><td>Thaw, mix, and briefly centrifuge each component before use.

Prior to use, dilute the Low Glycerol LibertyTaq™ DNA Polymerase (50 U/µL) to at least 10 U/µL in Low Glycerol LibertyTaq™ Diluent included in the kit.

Note: After dilution, the Low Glycerol LibertyTaq™ DNA Polymerase (at 10 U/µL) can be stored at 4°C for use within 1–2 days. Do not store the diluted enzyme at –20°C.</td></tr>
<tr><td>2  </td><td>Prepare qPCR master mix</td><td>Add the following components to each qPCR reaction 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>
<tr><th>Component</th><th>25-µL rxn</th><th>Custom</th><th>Final conc.</th></tr>
<tr><td>Water, nuclease-free</td><td>to 25 µL</td><td>µL</td><td>—</td></tr>
<tr><td>20X Liberty PCR Buffer (– $MgCl_2$)</td><td>1.25 µL</td><td>µL</td><td>1X</td></tr>
<tr><td>50 mM $MgCl_2$</td><td>0.75 µL</td><td>µL</td><td>1.5 mM</td></tr>
<tr><td>10 mM dNTP mix</td><td>0.5 µL</td><td>µL</td><td>0.2 mM each</td></tr>
<tr><td>100 µM primer probe</td><td>0.05 µL</td><td>µL</td><td>0.2 µM</td></tr>
<tr><td>30 µM ROX Reference Dye</td><td>0.025 µL</td><td>µL</td><td>30 nM</td></tr>
<tr><td>Low Glycerol LibertyTaq™ DNA Polymerase (10 U/µL)</td><td>0.1 µL</td><td>µL</td><td>1 U/rxn</td></tr>
</table>

Mix and then briefly centrifuge the components.</td></tr>
<tr><td>3  </td><td>Add template DNA and primers</td><td>Add your template DNA and primers to each tube for a final reaction volume of 25 µL.

<table>
<tr><th>Component</th><th>25-µL rxn</th><th>Custom</th><th>Final conc.</th></tr>
<tr><td>10 µM forward gene-specific primer</td><td>0.75 µL</td><td>µL</td><td>0.3 µM</td></tr>
<tr><td>10 µM reverse gene-specific primer</td><td>0.75 µL</td><td>µL</td><td>0.3 µM</td></tr>
<tr><td>Template DNA</td><td>varies</td><td>µL</td><td>≤250 ng/rxn (human gDNA)[1]</td></tr>
</table>
[1] See “Important guidelines”, page 1.

Cap each tube, mix, and then briefly centrifuge the contents.</td></tr>
</table>

Steps	Action	Procedure details
4 	**Incubate reactions in a thermal cycler**	qPCR cycling parameters (Two-step cycling protocol):

Step		Temperature	Time
Initial denaturation		95°C	5 minutes
40 qPCR cycles	Denature	95°C	15 seconds
	Anneal/Extend[1]	~60°C (depending on primer T_m)	1 minute

[1] Data acquisition should be performed during the annealing/extension step for probe-based assays.

qPCR cycling parameters (Three-step cycling protocol):

Step		Temperature	Time
Initial denaturation		95°C	5 minutes
40 qPCR cycles	Denature	95°C	15 seconds
	Anneal	~60°C (depending on primer T_m)	30 seconds
	Extend[1]	72°C	30 seconds

[1] Data acquisition should be performed during the extension step for probe-based assays.

5 	**Analyze results**	▪ Analyze results following your real-time instrument manufacturer's guidelines. ▪ 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.