Invitrogen™ Low Glycerol Platinum™ *Taq*, CG DNA Polymerase

invitrogen

USER GUIDE

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Print Options

Package contents

Catalog number	Size	
EP147B002	2500 rxns	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)
- Water, nuclease-free
- 0.2 or 0.5-mL nuclease-free microcentrifuge tubes
- *for qPCR*:
  100 µM primer probe
  Invitrogen™ ROX Reference Dye (Cat. no. 12223-012)
- *for PCR*:
  Invitrogen™ E-Gel™ General Purpose Gels, 1.2% (Cat. no. G5018-01)
  Invitrogen™ TrackIt™ 1 kb Plus DNA Ladder (Cat. no. 10488-085)

Timing

Varies depending on amplicon length

Product description

- 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.

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

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-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 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 µL per well.

Step	Action	Procedure details
1 	**Thaw reagents**	Thaw, mix, and briefly centrifuge each component before use. 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. **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.
2 	**Prepare PCR master mix**	Add the following components to each PCR 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. **Component** / **50-µL rxn** / **Custom** / **Final conc.** Water, nuclease-free / to 50 µL / 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 Low Glycerol Platinum™ *Taq*, CG DNA Polymerase (10 U/µL) / 0.2 µL / µL / 2 U/rxn Mix and then briefly centrifuge the components.
3 	**Add template DNA and primers**	Add your template DNA and primers to each tube for a final reaction volume of 50 µL. **For qPCR:** **Component** / **50-µL rxn** / **Custom** / **Final conc.** 10 µM forward gene-specific primer / 1.5 µL / µL / 0.3 µM 10 µM reverse gene-specific primer / 1.5 µL / µL / 0.3 µM 100 µM primer probe / 0.1 µL / µL / 0.2 µM 30 µM ROX Reference Dye[1] / 0.05 µL / µL / 30 nM Template DNA / varies / µL / ≤500 ng/rxn (human gDNA)[1] [1] See "Optimization strategies", page 1. **For PCR:** **Component** / **50-µL rxn** / **Custom** / **Final conc.** 10 µM forward gene-specific primer / 1 µL / µL / 0.2 µM 10 µM reverse gene-specific primer / 1 µL / µL / 0.2 µM Template DNA / varies / µL / ≤500 ng/rxn (human gDNA)[1] [1] See "Optimization strategies", page 1. Cap each tube, mix, and then briefly centrifuge the contents.

Step	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
25–40 PCR cycles	Denature	95°C	15 seconds[1]
	Anneal/Extend[2]	~60°C (depending on primer T_m)	1 minute

[1] If amplicon is ≥1500 bp, increase the denaturation time to 30 seconds.
[2] 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
25–40 PCR cycles	Denature	95°C	15 seconds[1]
	Anneal	~60°C (depending on primer T_m)	30 seconds
	Extend[2]	72°C	30 seconds

[1] If amplicon is ≥1500 bp, increase the denaturation time to 30 seconds.
[2] Data acquisition should be performed during the extension step for probe-based assays.

PCR cycling parameters:

Step		Temperature	Time
Initial denaturation		95°C	5 minutes
25–40 PCR cycles	Denature	95°C	15 seconds[1]
	Anneal	~60°C (depending on primer T_m)	30 seconds
	Extend	72°C	1 minute/kb
Hold		4°C	indefinitely

[1] If amplicon is ≥1500 bp, increase the denaturation time to 30 seconds.

5 	**Analyze results**	▪ 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.