Platinum™ *Taq* DNA Polymerase, DNA-free

USER GUIDE Pub. No. MAN0018458 Rev. A.0

invitrogen

Print Options

Contents and storage

Catalog No.	Amount	Storage
15966005 15966025	500 U 5 × 500 U	–20°C

Kit contents

Product description

- The Invitrogen™ Platinum™ *Taq* DNA Polymerase, DNA-free, is an ideal choice for PCR-based applications requiring the highest sensitivity without false-positive results from reagent-borne contamination.
- Platinum™ *Taq* DNA Polymerase, DNA-free, is manufactured using closed and single-use system technology to minimize DNA contamination risk. It contains ≤0.01 genome equivalent of bacterial DNA and ≤0.001 copy of human genomic DNA per enzyme unit. Go to thermofisher.com/dna-free for additional information.
- The enzyme is a recombinant *Taq* polymerase complexed with a proprietary antibody that blocks polymerase activity at ambient temperatures.
- Activity is restored after the initial 94°C denaturation step during PCR, providing an automatic "hot start" and offering increased sensitivity, specificity, and yield, while allowing reaction assembly at room temperature.

Required materials

- Template: cDNA, gDNA
- Forward and reverse gene-specific primers
- 10 mM dNTP mix (Cat. No. 18427-088)
- Water, nuclease-free
- 0.2-mL or 0.5-mL nuclease-free microcentrifuge tubes

for PCR:

- E-Gel™ General Purpose Gels, 1.2% (Cat. No. G5018-01)
- TrackIt™ 1 kb Plus DNA Ladder (Cat. No. 10488-085)

for qPCR:

- 100 µM qPCR probe ROX Reference Dye

Online resources

- Visit our product page for protocols, safety, and additional product information.
- For support, visit thermofisher.com/support.

Important guidelines

IMPORTANT: Always maintain a DNA-free environment when handling vials with polymerase and other PCR reagents to prevent DNA contamination.

Click here for important PCR guidelines.

Enzyme characteristics

Hot-start:	Antibody
Amplicon size:	Up to 4 kb
Fidelity vs. *Taq*:	1X
Product overhang:	3′ A
Exonuclease activity:	5′ → 3′

PCR setup

Use the following volumes to prepare your PCR reaction, or enter your own parameters in the column provided. For qPCR set-up, see page 2.

Component	25-µL rxn	50-µL rxn	Custom	Final conc. in 25-µL rxn
Water, nuclease-free	to 25 µL	to 50 µL	to.....µL	—
10X PCR Buffer (– $MgCl_2$), DNA-free	2.5 µL	5 µL	µL	1X
50 mM $MgCl_2$, DNA-free	0.75 µL	1.5 µL	µL	1.5 mM
10 mM dNTP mix	0.5 µL	1 µL	µL	0.2 mM each
10 µM forward primer	0.5 µL	1 µL	µL	0.2 µM
10 µM reverse primer	0.5 µL	1 µL	µL	0.2 µM
Template DNA	varies	varies	µL	≤500 ng/rxn
Platinum™ *Taq* DNA Polymerase, DNA-free (5 U/µL)	0.25 µL	0.5 µL	µL	1.25 U/rxn

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-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 1 — Thaw reagents

a. UV irradiate your work area prior to beginning work to ensure a DNA-free environment.

b. Thaw, mix, and briefly centrifuge each component before use.

Step 2 — Prepare PCR master mix

a. 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	25-µL rxn	Custom	Final conc.
Water, nuclease-free	to 25 µL	µL	—
10X PCR Buffer (– $MgCl_2$), DNA-free	2.5 µL	µL	1X
50 mM $MgCl_2$, DNA-free	0.75 µL	µL	1.5 mM
10 mM dNTP mix	0.5 µL	µL	0.2 mM each
Platinum™ *Taq* DNA Polymerase, DNA-free (5 U/µL)	0.25 µL	µL	1.25 U/rxn

b. Mix and then briefly centrifuge the components.

Step 3 — Add template DNA and primers

a. Add your template DNA and primers to each tube for a final reaction volume of 25 µL.

For PCR[1]:

Component	25-µL rxn	Custom	Final conc.
10 µM forward gene-specific primer	0.5 µL	µL	0.2 µM
10 µM reverse gene-specific primer	0.5 µL	µL	0.2 µM
Template DNA	varies	µL	≤500 ng/rxn (human gDNA)

[1] See "Optimization strategies", page 1.

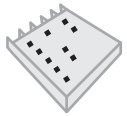
For qPCR[1]:

Component	25-µL rxn	Custom	Final conc.
10 µM forward gene-specific primer	0.75 µL	µL	0.3 µM
10 µM reverse gene-specific primer	0.75 µL	µL	0.3 µM
100 µM qPCR probe	0.05 µL	µL	0.2 µM
30 µM ROX Reference Dye	0.025 µL	µL	30 nM[2]
Template DNA	varies	µL	≤500 ng/rxn (human gDNA)

[1] See "Optimization strategies", page 1.

[2] The recommended final ROX concentration depends on the instrument (see "Important guidelines", page 1).

b. Cap each tube, mix, and then briefly centrifuge the contents.

Step	Action	Procedure details
4 	**Incubate reactions in a thermal cycler**	**PCR cycling parameters:** **Step** / **Temperature** / **Time** Initial denaturation — 94°C — 2 minutes 25–40 PCR cycles: Denature — 94°C — 30 seconds 25–40 PCR cycles: Anneal — ~60°C (depending on primer T_m) — 30 seconds 25–40 PCR cycles: Extend — 72°C — 1 minute/kb Hold — 4°C — hold **qPCR cycling parameters (Two-step cycling protocol):** **Step** / **Temperature** / **Time** Initial denaturation — 94°C — 2 minutes 25–40 PCR cycles: Denature — 94°C — 30 seconds 25–40 PCR cycles: 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 — 94°C — 2 minutes 25–40 PCR cycles: Denature — 94°C — 30 seconds 25–40 PCR cycles: Anneal — ~60°C (depending on primer T_m) — 30 seconds 25–40 PCR cycles: Extend[1] — 72°C — 30 seconds [1] Data acquisition should be performed during the extension step for probe-based assays.
5 	**Analyze results**	**a.** 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. **b.** Analyze qPCR results following your real-time instrument manufacturer's guidelines. **c.** You can store your samples overnight at 2–8°C, or at –20°C for longer period.