Invitrogen™ Lyo-ready Platinum™ II *Taq* Hot-Start DNA Polymerase

invitrogen

USER GUIDE

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Print Options

Package contents

Sample Kit No.	Size
EP204SMP	1 kU

Kit contents

Storage conditions

Store all contents at –20°C. Product is designed to withstand at least 10 freeze-thaw cycles.

Required materials

- Template: cDNA, genomic DNA, plasmid DNA, phage DNA
- Forward and reverse primers
- qPCR probe
- Water, nuclease-free
- 0.2-mL or 0.5-mL nuclease-free microcentrifuge tubes

Timing

Varies depending on amplicon length.

Product description

- Invitrogen™ Lyo-ready Platinum™ II *Taq* Hot-Start DNA Polymerase is an engineered *Taq* DNA polymerase that shows increased resistance to reaction inhibitors originating from sample material or DNA purification steps. The lyo-ready enzyme formulation combines feasibility to lyophilize, while retaining all favourable standard enzyme (with glycerol) properties.
- The polymerase activity is blocked at ambient temperatures and restored after the initial denaturation step at 95°C. This automatic "hot start" provides increased sensitivity, specificity, and yield, while allowing reaction assembly at room temperature.
- Lyo-ready Platinum™ II *Taq* Hot-Start DNA Polymerase extends 1 kb in 15 seconds. The extension step can be prolonged without a negative effect on specificity.
- The enzyme has a template independent 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 activities, but lacks 3′ to 5′ exonuclease activity.

Online resources

Find out more at Lyo-ready PCR Enzymes.

For further information, contact LCSVilnius@thermofisher.com.

Enzyme characteristics

Hot-start: Antibody

Fidelity vs. *Taq*: 1X

Format: Separate components

Experiment setup

Use the following amounts to prepare your qPCR experiment. Note that the table shows the appropriate volumes for a single **25-µL** reaction.

Component	Volume	Final conc.
Water, nuclease-free	to 25 µL	—
5X Lyo-ready Platinum™ II PCR Buffer 2, w/o $MgCl_2$	5 µL	1X
10 mM dNTP mix	0.5 µL	0.2 mM each
$MgCl_2$ (50 mM)	0.75 µL	1.5 mM
10 µM forward primer	0.75 µL	0.3 µM
10 µM reverse primer	0.75 µL	0.3 µM
10 µM primer probe	0.5 µL	0.2 µM
(*Optional*) ROX Reference Dye[1]	varies	varies
Template DNA[1]	varies	≤500 ng/rxn
Lyo-ready Platinum™ II *Taq* Hot-Start DNA Polymerase (5 U/µL)	0.2 µL	1 U/rxn

[1] See "Optimization strategies", below.

Protocol

Go to page 2 for instructions to prepare and run your qPCR experiment.

Optimization strategies

Click here for guidelines to optimize your qPCR experiment.

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The following example procedure shows the appropriate volumes for a single **25-µL** qPCR reaction. For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, then dispense the appropriate volumes into each 0.2-mL or 0.5-mL PCR tube or well of a MicroAmp™ EnduraPlate™ Optical 96-well Fast Plate before adding template DNA and primers.

Steps | Action | Procedure details

1 Thaw reagents

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

2 Prepare PCR master mix

a. Add the following components to each 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.

Component	Volume for 25-µL rxn	Final concentration
Water, nuclease-free	to 25 µL	—
5X Lyo-ready Platinum™ II PCR Buffer 2, w/o $MgCl_2$	5 µL	1X
10 mM dNTP mix (10 mM each)	0.5 µL	0.2 mM each
$MgCl_2$ (50 mM)	0.75 µL	1.5 mM
Lyo-ready Platinum™ II *Taq* Hot-Start DNA Polymerase	0.2 µL	0.04 U/µL

b. Mix, then briefly centrifuge the components.

3 Add template DNA and primers

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

Component	Volume for 25-µL rxn	Final concentration
10 µM forward primer	0.75 µL	0.3 µM
10 µM reverse primer	0.75 µL	0.3 µM
10 µM primer probe	0.5 µL	0.2 µM
(*Optional*) ROX Reference Dye	varies	varies
Template DNA	varies	<500 ng/rxn

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

4 Incubate reactions in a thermal cycler

Step		Temperature	Time
Initial denaturation		95°C	2 minutes
40 cycles	Denature	95°C	5 seconds
	Anneal/Extend[1]	60°C	15 seconds

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

Note: Refer to "Optimization strategies", page 1, for guidelines to optimize cycling conditions.