Invitrogen™ Platinum™ Hot Start PCR 2X Master Mix

Pub. no. MAN0014007 Rev. A.0

Package contents

Catalog number	Size
13000-012	50 rxns
13000-013	200 rxns
13000-014	1000 rxns

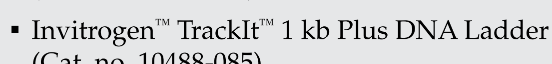
Kit contents

Storage conditions

- Store all contents at –20°C.

Required materials

- Template: cDNA, gDNA, λDNA
- Forward and reverse gene-specific primers
- Invitrogen™ E-Gel™ General Purpose Gels, 1.2% (Cat. no. G5018-01)
- Invitrogen™ TrackIt™ 1 kb Plus DNA Ladder (Cat. no. 10488-085)



- 0.2 or 0.5-mL nuclease-free microcentrifuge tubes
- Gel loading buffer

Timing

Varies depending on amplicon length

Selection guide

PCR Enzymes and Master Mixes
Go online to view related products.

Product description

- Platinum™ Hot Start PCR 2X Master Mix contains Platinum™ *Taq* DNA Polymerase in an optimized PCR buffer with Mg^{2+} and dNTPs.
- The master mix retains all features of the Platinum™ *Taq* DNA Polymerase.
- Specific binding of the anti-*Taq* antibody inhibits polymerase activity at ambient temperatures. Activity is restored after the initial denaturation step in PCR cycling at 94°C, providing an automatic "hot start" and offering increased sensitivity, specificity, and yield, while allowing reaction assembly at room temperature.
- Platinum™ GC Enhancer improves amplification of GC-rich targets.

Important guidelines

Click here for important PCR guidelines.

Online resources

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

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

Enzyme characteristics

Hot-start: Antibody
Length: Up to 5 kb
Fidelity vs. *Taq*: 1X
Format: Master mix

PCR setup

Use the measurements below to prepare your PCR experiment, or enter your own parameters in the column provided.

Component	25-µL rxn	50-µL rxn	Custom	Final conc. in 50-µL rxn
Water, Nuclease-free	to 25 µL	to 50 µL	to µL	—
Platinum™ Hot Start PCR 2X Master Mix	12.5 µL	25 µL	µL	1X*
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		<500 ng/rxn
Platinum™ GC Enhancer (*optional*)**	5 µL	10 µL	µL	20%

* 1X master mix contains a final concentration of 1.5 mM $MgCl_2$ and 0.2 mM of each dNTP.
** Recommended only for targets with >65% GC sequences.

PCR protocol

See page 2 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 PCR procedure below shows appropriate volumes for a single **50-µL** reaction using GC-rich template DNA. For multiple reactions, prepare a master mix of common reaction components, then dispense appropriate volumes into each 0.2–0.5 mL PCR tube prior to adding template DNA and primers.

Steps	Action	Procedure details
1	**Thaw reagents**	Thaw, mix, and briefly centrifuge each component before use. Avoid generating bubbles when mixing the Master Mix.
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. (see table below) *For targets with >65% GC sequences. 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. (see table below) Cap each tube, mix, and then briefly centrifuge the contents.
4	**Incubate reactions in a thermal cycler**	(see table below)
5	**Add gel loading buffer and analyze with gel electrophoresis**	Add gel loading buffer to 10 µL of PCR sample, mix, and briefly centrifuge the contents. Analyze the sample using agarose gel electrophoresis. Use your PCR product immediately in down-stream applications, or store it at –20°C.

Step 2:

Component	50-µL rxn	Final conc.
Water, Nuclease-free	to 50 µL	—
Platinum™ Hot Start PCR 2X Master Mix	25 µL	1X
Platinum™ GC Enhancer (*optional*)*	10 µL	20%

*For targets with >65% GC sequences.

Step 3:

Component	50-µL rxn	Final conc.
10 µM forward primer	1 µL	0.2 µM
10 µM reverse primer	1 µL	0.2 µM
Template DNA	varies	<500 ng/rxn

Step 4:

Step		Temperature	Time
Initial denaturation		94°C	2 minutes
25–35 PCR cycles	Denature	94°C	30 seconds
	Anneal	~55°C (depending on primer T_m)	30 seconds
	Extend	72°C	1 minute/kb
Hold		4°C	indefinitely