# Invitrogen™ Platinum™ SuperFi™ DNA Polymerase

invitrogen

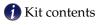
**USER GUIDE** 

Pub. no. MAN0014882 Rev. A.0



## Package contents

Catalog number Size 12351-010 100 Units 12351-050 500 Units 12351-250  $5 \times 500$  Units





#### Storage conditions

- Store all contents at -20°C.
- Template: gDNA, plasmid DNA, phage DNA, cDNA
- Forward and reverse gene-specific primers
- Invitrogen<sup>™</sup> 10 mM dNTP Mix (Cat. no. 18427-088)
- Invitrogen<sup>™</sup> E-Gel<sup>™</sup> General Purpose Gels, 1.2% (Cat. no. G5018-01)



- Invitrogen<sup>™</sup> TrackIt<sup>™</sup> 1 kb Plus DNA Ladder (Cat. no. 10488-085)
- 0.2 or 0.5-mL nuclease-free microcentrifuge tubes
- Gel loading buffer
- Water, nuclease-free



#### Timing

Varies depending on amplicon length.



#### Selection auide

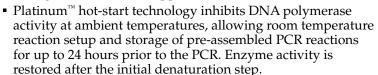
Product

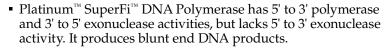
description

PCR Enzymes and Master Mixes

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 Platinum<sup>™</sup> SuperFi<sup>™</sup> DNA Polymerase is a proofreading DNA polymerase that combines superior fidelity with Platinum<sup>™</sup> hot-start technology, and is ideally suited for cloning, mutagenesis, and other applications.





 Platinum<sup>™</sup> SuperFi<sup>™</sup> DNA Polymerase is supplied with a separate vial of SuperFi<sup>™</sup> GC Enhancer designed for GC-rich templates (>65% GC).

# Important guidelines

**(1)** Click here for important PCR guidelines.



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

# **Enzyme characteristics**

**Hot-start:** Antibody Length: Up to 20 kb

>100XFidelity vs. *Taq*:

Format: Separate components

# PCR setup

| Component   | 25-μL rxn | 50-μL rxn    | Custom | Final conc. |  |
|---|-----------|--------------|--------|-------------|--|
| Water, nuclease-free  | to 25 µL  | to 50 µL     | to µL  | _           |  |
| 5X SuperFi™ Buffer¹   | 5 μL      | 10 μL        | μL     | 1X          |  |
| 10 mM dNTP mix  | 0.5 μL    | 5 μL 1 μL μL |        | 0.2 mM each |  |
| 10 μM forward primer  | 1.25 µL   | 2.5 µL       | μL     | 0.5 μΜ      |  |
| 10 μM reverse primer  | 1.25 μL   | 2.5 µL       | μL     | 0.5 μΜ      |  |
| Template DNA <sup>2</sup>                                   | varies    | varies       |        | varies      |  |
| 5X SuperFi <sup>™</sup> GC Enhancer (optional) <sup>3</sup> | 5 μL      | 10 μL        | μL     | 1X          |  |
| Platinum™ SuperFi™ DNA<br>Polymerase (2 U/ μL)              | 0.25 μL   | 0.5 µL       | μL     | 0.02 U/μL   |  |

<sup>&</sup>lt;sup>1</sup> Includes 7.5 mM MgCl<sub>2</sub>.

## PCR protocol

1 See page 2 and page 3 to prepare and run your PCR experiment.

## Optimization strategies and troubleshooting

- n Click here for guidelines to optimize your PCR experiment.
- 1 Click here for guidelines to troubleshoot your PCR experiment.

## Purchaser notification

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<sup>&</sup>lt;sup>2</sup> 5–50 ng gDNA or 1 pg–10 ng plasmid DNA (see "**Optimization strategies**", below, for more information).

<sup>&</sup>lt;sup>3</sup> Recommended for targets with >65% GC sequences.

The example PCR procedure below shows appropriate volumes for a single  $50-\mu L$  reaction. For multiple reactions, prepare a master mix of components common to all reactions to minimize pipetting error, 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.   |                   |             |   |  |  |
|   |       | 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  Final conc.  |                   |             |   |  |  |
|   |       |                              | Water, nuclease-free 5X SuperFi™ Buffer¹   | to 50 μL<br>10 μL | 1X          | _ |  |  |
| 2 |       |                              | 10 mM dNTP mix   | 10 μL             | 0.2 mM each | _ |  |  |
|   |       |                              | 5X SuperFi™ GC Enhancer (optional)²  | 10 μL             | 1X          | _ |  |  |
|   |       |                              | Platinum <sup>™</sup> SuperFi <sup>™</sup> DNA Polymerase  | 0.5 μL            | 0.02 U/µL   | - |  |  |
|   |       |                              | <sup>1</sup> Includes 7.5 mM MgCl <sub>2</sub> . <sup>2</sup> Recommended for targets with >65% GC sequences.  Mix and then briefly centrifuge the components.   |                   |             |   |  |  |
|   |       | Add template DNA and primers | Add your template DNA and primers to each tube for a final reaction volume of 50 µL.   |                   |             |   |  |  |
| 3 |       |                              | Component  | 50-µL rxn         | Final conc. |   |  |  |
|   | 1 😝 1 |                              | 10 μM forward primer   | 2.5 µL            | 0.5 μΜ      |   |  |  |
|   | 8     |                              | 10 μM reverse primer   | 2.5 μL            | 0.5 μΜ      |   |  |  |
|   |       |                              | Template DNA <sup>1</sup>  | varies            | varies      |   |  |  |
|   |       |                              | <sup>1</sup> Optimal amount of low complexity DNA (plasmid, phage, BAC DNA) is 1 pg–10 ng per 50 μL reaction, but it can be varied from 0.1 pg to 50 ng per 50 μL reaction. Optimal amount of genomic DNA is 5–50 ng per 50 μL reaction, but it can be varied from 0.1 ng to 250 ng per 50 μL reaction.  Cap each tube, mix, and then briefly centrifuge the contents. |                   |             |   |  |  |

| Steps | Action  | Procedure details   |                               |  |                   |   |  |  |   |
|-------|---|---|-------------------------------|--|-------------------|---|--|--|---|
| 4     | Incubate reactions in a<br>thermal cycler                   | Initial d  25–35 PCR cycles Final ex  1 IMPORT your prime   | ANT! Always ters and the reco | Temp. $98^{\circ}$ C $98^{\circ}$ C $ 72^{\circ}$ C $4^{\circ}$ C  use the $T_{m}$ calcummended annotation | nealing temperatu | Temp. 98°C 98°C varies 72°C 72°C 4°C site at www.th | tocol (<10kb)  Time 30 sec 5–10 sec 10 sec 15–30 sec/kb 5 min hold ermofisher.com/tr | Temp.  95°C  95°C  varies  68°C  68°C  4°C  mcalculator to | Time 2 min 10 sec 10 sec 30 sec/kb 5 min hold calculate the T <sub>m</sub> of |
| 5     | Add gel loading buffer and analyze with gel electrophoresis | Add gel loading buffer to 10 µL of PCR product, 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. |                               |  |                   |   |  |  |   |

