



i7[®] High-Fidelity DNA Polymerase 2x Master Mix

Manual

| | | |
|---------------------|---------------|---------------|
| Catalog # | 3257 | 3259 |
| Package Size | 100 reactions | 500 reactions |



Important!

-20°C Storage Required

- * Immediately inspect packages
- * Freeze upon receipt



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Intact Genomics, Inc.

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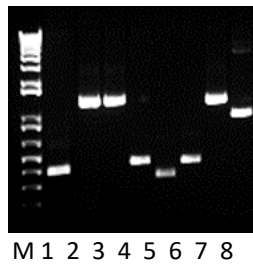
Description:

Intact Genomics (ig®) i7® High-Fidelity DNA Polymerase 2x Master Mix is ready to use premix which contains i7® highfidelity DNA Polymerase, dNTPs, MgCl₂, PCR enhancers and stabilizers with optimized proprietary reaction buffer. i7 high-fidelity DNA Polymerase is a genetically engineered, heat stable DNA polymerase which has 5'→3' polymerase and 3'→5' exonuclease (proofreading) activities. This enzyme has high-fidelity, sensitivity and processivity with an error rate ~2.8x10⁻² -fold lower than *Taq* DNA polymerase, and significantly lower than other proofreading enzymes in the marketplace (1). Proprietary buffer allows for amplification of non GC rich templates and of GC rich templates up to 84%.

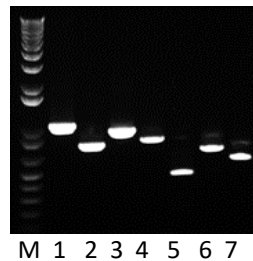
Activity Data:

We have tested i7® High-Fidelity 2x Master Mix with difficult templates for PCR amplification. Typical PCR results are shown below:

A). Colony PCR



B). PCR to detect difficult templates



Applications:

- Long and difficult template DNA amplification
- Cloning
- High-fidelity PCR

Product Components:

- i7® High-Fidelity DNA Polymerase 2x Master Mix

Storage Temperature and Buffer:

- -20°C
- 50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.5 @ 25°C

Heat Inactivation:

No

Protocol:

1. Thaw i7® high-fidelity 2x master mix and primer solutions and mix thoroughly before use.
2. Prepare a reaction mix according to the following table: (The reaction mix typically contains all the components needed for PCR except the template DNA).

| Components | 20 µl reaction | 50 µl reaction | Final concentration |
|---------------------------------|----------------|----------------|---------------------|
| Template DNA | variable | variable | 1-1000 ng |
| Forward primer (10 µM) | 1.0 µl | 2.5 µl | 0.5 µM |
| Reverse primer (10 µM) | 1.0 µl | 2.5 µl | 0.5 µM |
| i7® high-fidelity 2x Master Mix | 10.0 µl | 25.0 µl | 1x |
| H ₂ O up to | 20.0 µl | 50.0 µl | |

3. Mix the reaction mixture thoroughly.
4. Add template DNA to the individual PCR tube containing the reaction mixture.
5. Program the thermal cycler according to the manufacturer's instructions. A typical PCR cycling program is outlined in the following table:

| PCR Cycling Conditions | | | |
|------------------------|----------|--------------|--------|
| Steps | Temp. | Time | Cycles |
| Initial denaturation | 98 °C | 1-2 min | 1 |
| Denaturation | 98 °C | 10-20 sec | 25-35 |
| Annealing | 52-66 °C | 10-30 sec | |
| Extension | 68-72 °C | 10-30 sec/kb | |
| Final extension | 68-72 °C | 5 min | 1 |
| Hold | 4-12 °C | ∞ | |

6. Place the PCR tubes in the thermal cycler and start the cycling program.

Related Products:

- *Taq* DNA Polymerase 2x Premix (Cat.# 3249)
- ig® 10B Electrocompetent Cells (Cat.# 1212-12)
- ig® 10B Chemically Competent Cells (Cat.# 1011-12)
- ig-Fusion™ Cloning Kit (Cat.# 4111)
- i7® Hot Start High-Fidelity DNA Polymerase (Cat.#3281)
- i7® High-Fidelity DNA Polymerase (Cat.#3254)

Ordering Information:

- Order online within the USA. Place orders on **www.intactgenomics.com** using our secure Shopping Cart.
- Order by email, phone, or fax.
Email: **sales@intactgenomics.com**
Phone: (314) 942-3655 | Toll-free : 855-835-7172 | Fax: (314) 942-3656
- Order via our distributors.

References:

1. Frey, B. and Suppman, B. (1995). BioChemica. 2, 34 -35.

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Our hours are Monday - Friday, 8AM to 5PM, U.S. Central Standard Time.

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