BIO-RAD

iProof™ High-Fidelity PCR Master Mix

Sso7d FUSION POLYMERASE
EMPOWER YOUR PCR

Catalog #	Description
1725310	**iProof HF Master Mix**, 100 x 50 µl reactions; 2x master mix 2 x 1.25 ml vials, 100% DMSO 1 x 0.5 ml vial
1725320	**iProof GC Master Mix**, 100 x 50 µl reactions; 2x master mix 2 x 1.25 ml vials, 100% DMSO 1 x 0.5 ml vial

For research purposes only.

Introduction

iProof High-Fidelity PCR Master Mixes are 2x concentrated, ready-to-use supermixes containing all components for high-fidelity, long, or fast PCR for amplicons up to 37 kb. Simply add your template and primers to start your reactions.

The iProof High-Fidelity DNA Polymerase in these master mixes incorporates a patented* Sso7d fusion protein technology for speed and accuracy, making this an ideal choice for applications that require high fidelity, such as cloning. The error rate of of the iProof Polymerase is 50-fold lower than that of *Thermus aquaticus* DNA polymerase and sixfold lower than that of *Pyrococcus furiosus* DNA polymerase. iProof DNA Polymerase exhibits 5' → 3' polymerase activity and 3' → 5' exonuclease activity and produces blunt-end amplification products.

Storage and Stability

iProof HF Master Mix is guaranteed for 12 months at –20°C and for 2 months at 4°C. iProof GC Master Mix is guaranteed for 6 months at –20°C and for 3 months at 4°C. Avoid repeated freeze/thaw cycles to maintain optimal performance.

Reaction Setup

1. Thaw supermix and other frozen reaction components to room temperature. Mix thoroughly, centrifuge briefly to collect solution at the bottom of tubes, and store on ice.
2. Prepare reaction mix on ice using PCR tubes according to the recommendations in Table 1. Scale all components proportionally according to number of samples and reaction volumes.

Table 1. Reaction setup.

Component	Volume per 50 µl Reaction	Final Concentration
iProof HF or GC Master Mix (2x)	25 µl	1x
Forward and reverse primers**	Variable	0.5 µM each primer
DNA template	Variable	Plasmid DNA, lambda, or BAC DNA: 10 ng–1 pg Genomic DNA: 500–50 ng
Nuclease-free H_2O	Variable	—
DMSO*** optional	1.5 µl	3%
Total reaction mix volume	**50 µl**	—

** Recommended final primer concentration is 0.5 µM, but it can range between 0.2 and 1.0 µM if needed.
*** DMSO can be added for GC-rich amplicons. See Notes about Reaction Components for more details.

3. Seal tubes and vortex 30 sec or more to ensure homogeneity of the reaction components. Spin the tubes to remove any air bubbles and collect the reaction mixture in the vessel bottom.
4. Program the PCR cycling protocol on the PCR instrument according to Table 2.

Table 2. Thermal cycling protocol.

Step	Temperature, C°	Time	Cycle
Initial denaturation	98	30 sec–3 min	1
Denaturation	98	5–10 sec	25–35
Annealing	45–72 (optimized temperature)	10–30 sec	
Extension	72	15–30 sec/kb	
Final extension	72	5–10 min	1

* U.S. patents 6,627,424; 7,541,170; 7,560,260.

Notes about Cycling Conditions

- **Denaturation** — template denaturation should be performed at 98°C. The high thermostability of iProof DNA Polymerase allows denaturation temperatures greater than 98°C to be used if needed. A 30 sec initial denaturation time is recommended, but this can be extended to 3 min for difficult DNA templates. Subsequent denaturation should be performed for 5–10 sec at 98°C
- **Annealing** — when using iProof Master Mix, a general rule is to anneal primers (>20 nt) for 10–30 sec at 3°C above the primer with the lowest melting temperature (T_m). Primer T_m should be calculated using the nearest neighbor method as results can vary significantly, depending on the method used. For primers ≤20 nt, use an annealing temperature equal to the primer with the lowest T_m
- **Extension** — template extension temperature must be 72°C and extension time depends on amplicon length (15–30 sec/kb) and complexity. Do not exceed 1 min per kb for amplicons that are >5 kb

Notes about Reaction Components

- **DNA polymerase** — the concentration of iProof DNA Polymerase in the iProof PCR Master Mixes is optimized to provide best results in most reactions. If reactions are set up according to the protocol listed, the final concentration of iProof DNA Polymerase in the final reaction is 1 unit per 50 µl. iProof DNA Polymerase generates blunt ends in PCR products. If cloning is the subsequent application, blunt-end cloning is recommended. If TA cloning is required, the PCR products should be purified prior to adding A overhangs, as iProof DNA Polymerase will degrade any overhangs generated
- **Buffers** — the iProof HF Master Mix contains iProof HF Buffer and the iProof GC Master Mix contains iProof GC Buffer. The error rate of iProof DNA Polymerase in HF buffer is 4.4×10^{-7}, which is lower than that in GC buffer (9.5×10^{-7}). Thus, the iProof HF Master Mix should be used as the default reagent for high-fidelity amplification and the iProof GC Master Mix can be used for difficult or long templates, such as GC-rich templates, or those with complex secondary structures. The iProof GC Master Mix should be used in reactions where the HF Master Mix does not provide satisfactory results
- **$MgCl_2$ and dNTPs** — the iProof PCR Master Mixes provide 1.5 mM $MgCl_2$ and 200 µM of each dNTP in the final reaction concentration
- **DMSO** — adding 3% DMSO aids in template denaturation for GC-rich templates. Further optimization of DMSO concentration should be made in 2% increments. If a high DMSO concentration is used, the annealing temperature should be lowered by 5.5–6.0°C

Related Products

Catalog #	Description
1861096	**T100™ Thermal Cycler**
TBS0201	**0.2 ml High-Profile 8-Tube PCR Strips without Caps**, clear
TBS1201	**0.2 ml High-Profile 12-Tube PCR Strips without Caps**, clear
HSS9601	**Hard-Shell® High-Profile Semi-Skirted 96-Well PCR Plates**, clear shell/clear well
MSB1001	**Microseal® 'B' PCR Plate Sealing Film**, adhesive, optical

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