BIO-RAD

iProof™ High-Fidelity Master Mix

Catalog #	Kit	Kit Contents	Kit Size
172-5310	iProof HF Master Mix	Master mix — 2.5 ml (2 x 1.25 ml vials)	100 x 50 µl reactions
172-5320	iProof GC Master Mix	DMSO (100%) — (1 x 0.5 ml vial)	

For research purposes only.

Storage and Stability

Guaranteed for six months in a constant temperature freezer at –20°C. For convenience, the mix can be refrozen or stored at 4°C for three months.

Kit Contents

iProof high-fidelity PCR master mixes are 2x concentrated, ready-to-use supermixes containing all components (0.04 U/µl iProof, 400 µM dNTPs, and 3 mM $MgCl_2$), except primers and template, for high-fidelity, long, or fast PCR for amplicons up to 37 kb.

The iProof high-fidelity DNA polymerase in these master mixes incorporates a patented fusion protein technology for speed and accuracy, making this an ideal choice for cloning. The error rate of iProof polymerase is determined to be 50-fold lower than that of *Thermus aquaticus*, and six-fold lower than that of *Pyrococcus furiosus*. iProof polymerase produces blunt-end DNA products.

Reaction Setup

1. Thaw supermix and other frozen reaction components at 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.

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 (0.2 to 1.0 µM)
DNA template	Variable	Plasmid DNA, lambda, or BAC DNA: 10 ng–1 pg Genomic DNA: 500–50 ng
H_2O	Variable	—
Total reaction mix volume	*50 µl*	—

* Scale all components proportionally according to sample number and reaction volumes.

3. Seal tubes and vortex 30 sec or more to ensure thorough mixing 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	Time	Cycle
Initial denaturation	98°C	30 sec–3 min	1
Denaturation	98°C	5–10 sec	25–35
Annealing	45–72°C (optimized temperature)	10–30 sec	
Extension	72°C	15–30 sec/kb	
Final extension	72°C	5–10 min	1

Notes about Cycling Conditions and Reaction Components

- For difficult DNA templates, a 3 min initial denaturation time is recommended
- 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
- Template extension temperature must be at 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
- The error rate in HF buffer is 4.4×10^{-7}, which is lower than the error rate in GC buffer (9.5×10^{-7})
- Adding 3% DMSO aids in template denaturation for GC-rich amplicons. Further optimization of DMSO should be made in 2% increments. Ten-percent DMSO will require lowering the annealing temperature by 5.5–6.0°C

Quality Control

iProof HF and GC master mixes demonstrate robust amplification of long amplicons. Stringent specifications are maintained to ensure lot-to-lot consistency.

To learn more about Bio-Rad's complete list of solutions for amplification, visit **www.bio-rad.com/amplification**.