For ordering information on larger pack sizes, or to learn more about Bio-Rad amplification reagents and instruments, visit www.bio-rad.com/amplification/

Bio-Rad Laboratories
2000 Alfred Nobel Drive, Hercules, CA 94547
510-741-1000

10002299 Rev B

BIO-RAD

iProof™ High-Fidelity Master Mix

HF Master Mix	100 reactions	172-5310
HF Master Mix	500 reactions	172-5311
GC Master Mix	100 reactions	172-5320
GC Master Mix	500 reactions	172-5321

For research purposes only
Store at -20°C

iProof is a high-fidelity DNA polymerase that offers extreme performance for all PCR applications. Incorporating an exciting new and patented technology, iProof DNA polymerase brings together a novel *Pyrococcus*-like enzyme with a processivity enhancing domain. This allows for the generation of long templates with an accuracy and speed previously unattainable with a single enzyme. The extreme fidelity of iProof makes it a superior choice for cloning. The error rate of iProof polymerase is determined to be 4.4 x 10^{-7} in iProof HF buffer, which is approximately 50-fold lower than that of *Thermus aquaticus*, and 6-fold lower than that of *Pyrococcus furiosus*.

The iProof™ High Fidelity PCR Master Mixes include polymerase, nucleotides, and optimized reaction buffers. Enough reagents are included for performing 100 or 500 x 50 µl reactions.

Storage and Stability

Store the iProof™ High-Fidelity Master Mix at -20°C in a constant temperature freezer. When stored under these conditions, the mix is stable for six months after the ship date.

Kit Contents

Reagent	100 Reactions	500 Reactions	Description
2X iProof Master Mix	2 x 1.25 ml	10 x 1.25 ml	Master Mix containing: • 2X HF or GC Buffer* • 0.04 U/µl iProof • 400 µM dNTP (each)
DMSO	500 µl	2 x 500 µl	100% DMSO solution

*Both HF and GC Buffers contain 3 mM $MgCl_2$, which supplies 1.5 mM $MgCl_2$ in the final reaction.

iProof DNA polymerase is unlike other enzymes. Please read the *QuickGuide* to modify your protocol for optimal results.

QuickGuide (See *Notes About Cycling Conditions* for details)

- Use 98°C for denaturation.
- Anneal at T_m +3°C (>20nt oligo).
- Use 15–30 sec/kb for extension times. Do not exceed 1 min/kb.
- iProof produces blunt end DNA products.

Reaction Setup

Important Note – Please Read Before Starting

Spin all tubes before opening to improve recovery. Reactions should be set up on ice. Pipet all components in the order given below.

Component	Volume for 50 µl reaction	Volume for 20 µl reaction	Final Conc.
2X iProof Master Mix	25 µl	10 µl	1X
Primer 1**	x µl	x µl	0.5 µM
Primer 2**	x µl	x µl	0.5 µM
DNA template	x µl	x µl	
Sterile H_20	x µl	x µl	
Total Volume	50 µl	20 µl	

** Recommended final primer concentration is 0.5 µM; can range between 0.2–1.0 µM.

Notes About Reaction Components

1. **iProof DNA Polymerase**
   The enzyme concentration in the iProof Master Mix is optimized to supply 1 U of enzyme in a 50 µl reaction (0.4 U in a 20 µl reaction).
2. **Buffers**
   Two buffers options are available for the iProof Master Mix, HF and GC Buffer. The error rate of iProof polymerase in HF buffer (4.4×10^{-7}) is lower than that in GC buffer (9.5×10^{-7}). Therefore, the HF Master Mix should be used as the default mix for high fidelity amplification. However, the GC Master Mix can improve iProof performance on certain difficult or long templates, i.e. GC rich templates or those with complex secondary structures. Only use GC Master Mix when amplification with HF buffer does not provide satisfactory results.
3. **Mg^{2+} and dNTP**
   The iProof Master Mixes are optimized to provide 1.5 mM $MgCl_2$ and 200 µM dNTP (each) in the final reaction.
4. **DNA Template**
   General guidelines are 1 pg–10 ng of DNA template in a 50 µl reaction for low complexity DNA (e.g. plasmid, lambda, or BAC DNA). For high complexity DNA (e.g. genomic DNA), 50–500 ng of template DNA should be used in a 50 µl reaction.
5. **PCR Additives**
   The recommended reaction conditions for GC-rich templates include the addition of 3% DMSO which aids in template denaturation. Further optimization of DMSO should be made in 2% increments. In some cases, DMSO may be used to help relax supercoiled plasmid DNA. High DMSO concentrations (10%) will require lowering the annealing temperature by 5.5–6.0°C. Other PCR additives such as formamide, glycerol, and betaine are also compatible with iProof.

Cycling Conditions

Important Note – Please Read

Due to the novel nature of iProof DNA polymerase, optimal reaction conditions may differ from standard PCR protocols. iProof works better at elevated denaturation and annealing temperatures due to higher salt concentration in the reaction buffer.

Typical Thermal Cycling Protocol

Cycle Step	Temp.	Time	Number of Cycles
Initial Denaturation	98°C	30 s	1
Denaturation	98°C	5–10 s	
Annealing	45–72°C	10–30 s	25–35
Extension	72°C	15–30 s / kb	
Final Extension	72°C	5–10 min	1

Notes About Cycling Conditions

1. **Denaturation**
   Template denaturation should be performed at 98°C. Due to the high thermostability of iProof, denaturation temperatures greater than 98°C can be used. A 30 s 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 s at 98°C.
2. **Annealing**
   When using iProof, a general rule is to anneal primers (>20 nt) for 10–30 s at +3°C above the primer with the lowest 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 $\leq$ 20 nt, use an annealing temperature equal to the primer with the lowest T_m.
3. **Extension**
   Template extension should be performed at 72°C and extension time depends on amplicon length and complexity. For low complexity DNA (e.g. plasmid, lambda, or BAC DNA) use 15 s per kb. For high complexity DNA (e.g. genomic DNA) use 30 s per kb. **Do not exceed 1 min per kb for amplicons that are >5 kb.**

Component Specifications

iProof™ High Fidelity PCR Master Mix

2X iProof Master Mix contains 0.04 U/µl iProof™ High Fidelity DNA Polymerase, 2X iProof HF or GC Buffer, and 400 µM dNTP (each)

Amplification Performance Testing

iProof Master Mixes are tested in PCR amplification of a 7.5 kb genomic DNA & 20 kb lambda DNA amplicon.

Enzyme Stability and Storage

Each lot of iProof Master Mix is tested for stability under normal storage conditions (-20°C). The iProof Master Mix is stable for six months from the packaging date when stored and handled properly. After thawing, the mix can be refrozen or stored at +4°C for three months.

Related Amplification Products From Bio-Rad Laboratories

Reagents for PCR or Real-Time PCR

iProof™ High-Fidelity Polymerase	172-5301
iProof High-Fidelity PCR Kit	172-5331
iTaq™ DNA Polymerase	170-8870
iTaq Supermix With ROX	170-8854
iTaq SYBR Green Supermix With ROX	170-8850
iQ™ Supermix	170-8860
iQ SYBR Green Supermix	170-8880
iScript™ cDNA Synthesis Kit	170-8890
iScript Select cDNA Synthesis Kit	170-8896
iScript One-Step RT-PCR Kit with SYBR Green	170-8892
iScript One-Step RT-PCR Kit for Probes	170-8894