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.**

Related Amplification Products From Bio-Rad Laboratories

Reagents for PCR or Real-Time PCR

iProof™ High-Fidelity DNA Polymerase	172-5301
iProof HF Master Mix	172-5310
iProof GC Master Mix	172-5320
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

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

10002300 Rev B

BIO-RAD

iProof™ High-Fidelity PCR Kit

2 units/µl, 25 µl	50U	172-5330
2 units/µl, 100 µl	200U	172-5331

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 4.4×10^{-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 Kit includes lambda DNA control template and primers for 1.3 kb and 10 kb positive control amplicons. Sufficient template is included for performing 20 x 50 µl or 50 x 20 µl reactions.

Storage and Stability

Store the iProof™ High-Fidelity PCR Kit at -20°C in a constant temperature freezer. When stored under these conditions, the polymerase is stable for one year after the ship date.

Kit Contents

Reagent	50U	200U	Description
iProof Polymerase	25 µl	100 µl	iProof™ High Fidelity DNA Polymerase, 2 units/µl
iProof HF Buffer	1.5 ml	3 x 1.5 ml	5X HF Buffer, 7.5 mM $MgCl_2$
iProof GC Buffer	1.5 ml	3 x 1.5 ml	5X GC Buffer, 7.5 mM $MgCl_2$
dNTP mix	100 µl	100 µl	dNTP solution, 10 mM each
$MgCl_2$	1.5 ml	1.5 ml	50 mM $MgCl_2$ solution
Control 1template	40 µl	40 µl	Control 1template, 0.5 ng/µl
1.3 kb primers	50 µl	50 µl	4 µM each
10 kb primers	50 µl	50 µl	4 µM each
DNA Standard	200 µl	400 µl	DNA size standard
DMSO	500 µl	500 µl	100% DMSO solution

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.
- Use iProof at 0.5–1.0 U per 50 µl reaction. Do not exceed 2 U/50 µl.
- Use 200 µM dNTPs. Do not use dUTP.
- 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. Always add iProof DNA Polymerase last to the reaction as primer degradation may occur in the absence of dNTPs. It is recommended that you prepare a master mix for the appropriate number of samples to be amplified.

Typical Reaction Setup

Component	Volume for 50 µl reaction	Volume for 20 µl reaction	Final Conc.
5X iProof HF Buffer*	10 µl	4 µl	1X
dNTP mix	1 µl	0.4 µl	200 µM each
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	
iProof DNA Polymerase	0.5 µl	0.2 µl***	0.02 U/µl
Total Volume	50 µl	20 µl	

* For difficult or GC-rich templates, 5X iProof GC Buffer can be used.
** Recommended final primer concentration is 0.5 µM; can range between 0.2–1.0 µM.
*** Enzyme should be diluted to avoid pipeting errors.

Control Template Reaction Setup

Component	Volume for 50 µl reaction	Volume for 20 µl reaction	Final Conc.
5X iProof HF Buffer	10 µl	4 µl	1X
dNTP mix	1 µl	0.4 µl	200 µM each
Primers *	2.5 µl	1 µl	0.2 µM
Control DNA Template	2 µl	0.8 µl	
Sterile H_20	34 µl	13.6 µl	
iProof DNA Polymerase	0.5 µl	0.2 µl**	0.02 U/µl
Total Volume	50 µl	20 µl	

* Either 1.3 kb or 10 kb primers
** Enzyme should be diluted to avoid pipetting errors.

Notes About Reaction Components

1. **iProof DNA Polymerase**
   The optimal amount of enzyme depends on the amount of template and the length of the PCR product. Usually 1 unit of iProof DNA polymerase per 50 µl reaction will give good results, but optimal amounts could range from 0.5–2 units per 50 µl reaction depending on amplicon length and difficulty. **Do not exceed 2 U/50 µl (0.04 U/µl), especially for amplicons that are > 5kb**.
2. **Buffers**
   Two buffers are provided: 5x iProof HF buffer and 5x iProof 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 buffer should be used as the default buffer for high fidelity amplification. However, the GC buffer can improve iProof performance on certain difficult or long templates, i.e. GC rich templates or those with complex secondary structures. Only use GC buffer when amplification with HF buffer does not provide satisfactory results.
3. **Mg^{2+} and dNTP**
   Mg^{2+} concentration is critical since iProof is a Mg^{2+}-dependent enzyme. Excessive Mg^{2+} stabilizes dsDNA, preventing complete denaturation, and can also promote inaccurate priming. Conversely, insufficient amounts of Mg^{2+} can lead to low product yield. The optimal Mg^{2+} concentration also depends on dNTP concentration, the specific DNA template and the sample buffer composition. The optimal Mg^{2+} concentration is 0.5 to 1 mM over the total dNTP concentration for standard PCR. For optimization, increase or decrease Mg^{2+} concentration in 0.2 mM increments.
   Only high quality dNTPs should be used. Use of dUTP or other dUTP-derivatives or analogs is not recommended. Due to the increased processivity of iProof, there is no advantage to increasing dNTP amounts. For optimal results, use 200 mM dNTPs.
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

Control Template (1.3 kb) Cycling Protocol (2-step)

Cycle Step	Temp.	Time	Number of Cycles
Initial Denaturation	98°C	1 min	1
Denaturation	98°C	5 s	
Annealing/Extension	72°C	20 s	25–35
Final Extension	72°C	10 min	1

Control Template (10 kb) Cycling Protocol (3-step)*

Cycle Step	Temp.	Time	Number of Cycles
Initial Denaturation	98°C	1 min	1
Denaturation	98°C	5 s	
Annealing	60°C	15 s	25–35
Extension	72°C	2 min 30 sec	
Final Extension	72°C	10 min	1

* Both control template reactions can be run using the 10 kb cycling protocol.