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BIO-RAD

iProof™ High-Fidelity DNA Polymerase

2 units/μl, 10 μl	20U	172-5300
2 units/μl, 50 μl	100U	172-5301
2 units/μl, 250 μl	500U	172-5302

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*.

Storage and Stability

Store iProof™ High-Fidelity DNA Polymerase 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	20U	100U	500U	Description
iProof Polymerase	10 μl	50 μl	250 μl	iProof™ High Fidelity DNA Polymerase, 2 units/μl
iProof HF Buffer	1.5 ml	1.5 ml	4 x 1.5 ml	5X HF Buffer, 7.5 mM MgCl ₂
iProof GC Buffer	1.5 ml	1.5 ml	4 x 1.5 ml	5X GC Buffer, 7.5 mM MgCl ₂
MgCl ₂	1.5 ml	1.5 ml	2 x 1.5 ml	50 mM MgCl ₂ solution
DMSO	500 μl	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 $T_m + 3^\circ\text{C}$ (>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.

Component	Volume for 50 µl reaction	Volume for 20 µl reaction	Final Conc.
5X iProof HF Buffer*	10 µl	4 µl	1X
10 mM 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 ₂ O	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.

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. Mg²⁺ and dNTP

Mg²⁺ concentration is critical since iProof is a Mg²⁺-dependent enzyme. Excessive Mg²⁺ stabilizes dsDNA, preventing complete denaturation, and can also promote inaccurate priming. Conversely, insufficient amounts of Mg²⁺ can lead to low product yield. The optimal Mg²⁺ concentration also depends on dNTP concentration, the specific DNA template and the sample buffer composition. The optimal Mg²⁺ concentration is 0.5 to 1 mM over the total dNTP concentration for standard PCR. For optimization, increase or decrease Mg²⁺ 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 µM 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

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 ≤ 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

Storage buffer

20 mM Tris-HCl (pH 7.4 at 25°C), 0.1 mM EDTA, 1 mM DTT, 100 mM KCl, 0.5% Tween 20, 0.5% Nonidet P 40, 200 µg/ml BSA and 50% Glycerol

Unit Definition

One unit is defined as the amount of enzyme required to incorporate 10 nmoles of dNTPs into acid-insoluble form at 74°C in 30 minutes under the stated assay conditions.

Enzyme Stability

Each lot of DNA polymerase is tested for stability under normal storage conditions (-20°C). Enzyme stability is monitored at regular intervals for a two year period after the original assay date.