A Powerful Bond

Bio-Rad's unique iProof™ high-fidelity DNA polymerase delivers unsurpassed speed and fidelity — with higher yields and fewer reaction failures — for all your PCR needs.

Fusing the double-stranded DNA binding protein Sso7d to iProof gives it a powerful sliding grip on the replicated DNA.

Tips for Successful Amplification

- Use iProof high-fidelity DNA polymerase at 0.5–10 U per 50 µl reaction; do not exceed 2 U per 50 µl reaction
- Use 15–30 sec/kb for extension; do not exceed 1 min/kb
- Use 200 µM dNTPs; do not use dUTP
- Use 98°C for denaturation (high-salt buffer)
- Anneal at T_m + 3°C (>20 nt) or use 2-step protocol
- Note: iProof DNA polymerase produces blunt-end products

Recommended Cycling Parameters

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

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Troubleshooting Tips

Observation	Comments and Suggestions
No product or low yield	• Repeat and make sure that there are no pipetting errors • Use fresh high-quality dNTPs (170-8874); do not use dNTP mix that contains dUTP • Lower annealing temperature • Lengthen extension time • Optimize enzyme concentration • Denaturation time may be too long or too short; optimize the denaturation time • Check condition of the primers • Check primer design
Generally applies to G+C-rich or supercoiled templates	• Titrate DMSO (2–8% final) in the reaction • Denaturation temperature may be too low (98°C is optimal for most templates) • Try using GC buffer if HF buffer has failed
Generally applies to templates >5 kb	• Sample concentration may be too low; use more template • Template DNA may be damaged; use carefully purified template • Increase number of cycles
Nonspecific products — High molecular weight smears	• Reduce enzyme concentration • Shorten extension time • Reduce number of cycles • Increase annealing temperature or try 2-step protocol • Vary denaturation temperature
Nonspecific products — Low molecular weight discrete bands	• Raise annealing temperature • Lower enzyme concentration • Optimize Mg^{2+} concentration • Titrate template amount • Lower primer concentration • Design new primers
Generally applies to templates >5 kb	• Optimize extension time