TOOLS Hotstart Polymerase

Cat. no. TTC-PA22

Storage: -20℃

Product Size:

10× Hotstart Taq Buffer (Mg^{2+} plus)	2 mL
dNTP mix (10 mM)	400 µL
Hotstart Taq Polymerase (5 U/µL)	100 µL

Introduction

TOOLS Hotstart Polymerase is a monoclonal antibody that binds to and inhibits the activity of Taq DNA polymerase. Because of its unique thermal stability and high affinity to Taq DNA polymerase, it blocks Taq DNA polymerase activity at temperatures up to 65°C but releases fully active polymerase after 30 s of heat treatment at 95°C. TOOLS Hotstart Polymerase DNA Polymerase is a recombination of Taq DNA polymerase containing TOOLS Hotstart Polymerase that blocks polymerase activity at ambient temperatures. After PCR denaturation at 95℃, activity is restored, thereby providing an automatic PCR "hot start" for Taq DNA polymerase that provides increased sensitivity, specificity, and yield. In addition, it allows for the assembly of reactions at room temperature. Using TOOLS Hotstart Polymerase reduces the PCR optimization requirements; reaction setup time, handing, and effort; and contamination risk. No modifications to PCR reactions or protocols are necessary. This product has 5'–3' polymerase and exonuclease activity and lacks a 3'–5' exonuclease proofreading function. The PCR products contain A at the 3'-end and can be directly cloned into TA vectors.

Unit Definition:

One unit (U) is defined as the amount of enzyme that incorporates 10 nmol of dNTPs into acid-insoluble products within 30 min at 74°C, when using activated salmon sperm DNA as the template or primer.

Primer design

1. Use C or G as the last base of the 3'-end of the primer.
2. Avoid continuous mismatching of the last eight bases of the 3'-end of the primer.
3. Avoid hairpin structures at the 3'-end of the primer.
4. The Tm of the primers should be within the range 55°C–65°C.
5. When calculating the Tm of the primers, the 5'-sequence should not be included.
6. The GC content of the primers should be between 40% and 60%.

7. The Tm and GC content of the forward and reverse primers should be as similar as possible.

Recommended PCR Reaction

ddH_2O	≤50 µL
10× Hotstart Taq Buffer (Mg^{2+} plus)[a]	5 µL
dNTP Mix (10 mM)	1 µL
5× PCR enhancer[b]	optional
DNA Template[b]	optional
Primer 1 (10 µM)	2 µL
Primer 2 (10 µM)	2 µL
Hotstart Taq Polymerase (5 U/µL)	1 µL

1. For most PCR amplifications, 1.5–2.0 mM Mg^{2+} is optimal. The final Mg^{2+} concentration of 1× Taq Buffer of 2.0 mM guarantees successful amplification of most amplicons. However, Mg^{2+} can be further optimized in 0.5- or 1-mM increments by using a 25 mM $MgCl_2$ solution.
2. b. A PCR enhancer can be used to improve the amplification efficiency of templates with complex secondary structures, such as GC-rich sequences. Please note that an excessively high concentration of the PCR enhancer may reduce the amplification fidelity. Thus, we recommend using it only when the GC content is higher than 60% and when regular amplification cannot be achieved under optimized conditions.

The recommended amount of DNA template for a 50-µL reaction is as follows:

Human Genomic DNA	1–500 ng
Bacterial Genomic DNA	1–100 ng
λ DNA	0.1–1 ng
Plasmid DNA	0.1–1 ng

PCR Setup

95°C	30 s (Predenaturation)	
95°C	30 s	30–35 cycles
55°C *	30 s	
72°C	60 s/kb	
72°C	7 min (final extension)	

*Annealing temperature is based on and typically 1°C–2°C lower than the Tm of the primer pair.

The product is for research purposes only and not for diagnostic or clinical use.