HotStart *Taq* DNA Polymerase (Mg^{2+} Plus Buffer)

Catalog: RK20601
Size: 1,000 U / 5,000 U / 10,000 U
Concentration: 5,000 U/ml
Components:

HotStart *Taq* DNA Polymerase (5,000 U/ml)	RM20301
10X PCR Reaction Buffer, Mg^{2+} plus	RM20101

Product Description

HotStart *Taq* DNA polymerase is an innovative antibody-modified thermostable enzyme. The activity of the enzyme is completely inactive at room temperature, avoiding non-specific amplification and primer dimers during the preparation of the sample. As the temperature rises for the first cycle reaction, the specificity of DNA amplification is increased. When heated to 95 ℃, the antibody bound to the enzyme was rapidly inactivated and did not affect the subsequent *Taq* DNA polymerase reaction. The enzyme has the advantages of good specificity, high sensitivity, good reproducibility and high amplification efficiency.

Product Source:
An *E. coli* strain that carries the *Taq* DNA Polymerase gene from *Thermus aquaticus* YT-1.

Unit Definition:
One unit is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid-insoluble material in 30 minutes at 75 ℃.

1X PCR Reaction Buffer, Mg^{2+} plus:
20 mM Tris-HCl, 10 mM $(NH_4)_2SO_4$, 10 mM KCl, 2 mM $MgSO_4$, 0.1% Triton X-100, pH 8.8 @ 25 ℃

Storage Temperature: -20 ℃

Storage Conditions:
10 mM Tris-HCl, 100 mM KCl, 1 mM DTT, 0.1 mM EDTA, 0.5% Tween 20, 0.5% NP-40, 50% Glycerol, pH 7.4 @ 25 ℃

Molecular Weight: Theoretical 94000 daltons

Heat Inactivation: No

5' - 3' Exonuclease: Yes

3' - 5' Exonuclease: No

Strand Displacement: +

Resulting Ends: Single-base 3 ´Overhangs

Error Rate: ~ $285x10^{-6}$ bases

Instructions

Take 25/50 μl system as an example.

Composition	25 μl	50 μl	Final Conc.
ddH_2O	to 25 μl	to 50 μl	
10×PCR Buffer	2.5 μl	5 μl	1X (Final 2 mM Mg^{2+})
10 mM dNTP	0.5 μl	1 μl	200 μM
Primer F (10μM)	0.5 μl	1 μl	0.2μM (0.05~1 μM)
Primer R (10μM)	0.5 μl	1 μl	0.2μM (0.05~1 μM)
Template DNA	Variable	Variable	<1 μg/50 μl
HotStart *Taq* DNA Polymerase	0.125 μl	0.25 μl	1.25 U/50 μl (0.25–2.5 U/50 μl)

Notes: Gently mix the reaction. Collect all liquid to the bottom of the tube by a quick spin if necessary. Overlay the sample with mineral oil if using a PCR machine without a heated lid.

Incubated in a thermocycler as the below program.

Temperature	Time	Cycles
95 ℃	5 min	1
95 ℃ 45-68 ℃ 68 ℃	15-30s 15-60s 1kb/min	25-40
68 ℃ 4-10 ℃	5min ∞	1

General Guidelines:

1. Template:

Use of high quality, purified DNA templates greatly enhances the success of PCR. Recommended amounts of DNA template for a 50 µl reaction are as follows:

DNA	Amount
Genomic	1 ng–1 µg
Plasmid or viral	1 pg–1 ng

2. Primers:

Oligonucleotide primers are generally 20–40 nucleotides in length and ideally have a GC content of 40–60%. Computer programs such as Primer3 can be used to design or analyze primers. The final concentration of each primer in a PCR may be 0.05–1 µM, typically 0.1–0.5 µM.

3. Mg++ and additives:

Mg++ concentration of 1.5–2.0 mM is optimal for most PCR products generated with HotStart *Taq* DNA Polymerase.

Amplification of some difficult targets, like GC rich sequences, may be improved with additives, such as DMSO or formamide.

4. Deoxynucleotides:

The final concentration of dNTPs is typically 200 µM of each deoxynucleotide.

5. HotStart *Taq* DNA Polymerase Concentration:

We generally recommend using HotStart *Taq* DNA Polymerase at a concentration of 25 units/ml (1.25 units/50 µl reaction). However, the optimal concentration of HotStart *Taq* DNA Polymerase may range from 5–50 units/ml (0.25–2.5 units/50 µl reaction) in specialized applications.

6. Denaturation:

During thermocycling a 5 min denaturation at 95℃ is recommended to release the hot start inhibitor from the enzyme.

7. Annealing:

The annealing step is typically 15–60 seconds. Annealing temperature is based on the Tm of the primer pair and is typically 45–68℃. Annealing temperatures can be optimized by doing a temperature gradient PCR starting 5℃ below the calculated T_m.

8. Extension:

The recommended extension temperature is 68℃. Extension times are generally 1 minute per kb. A final extension of 5 minutes at 68℃ is recommended.

9. Cycle number:

Generally, 25–35 cycles yield sufficient product. Up to 45 cycles may be required to detect low-copy-number targets.

10. 2-step PCR:

When primers with annealing temperatures above 68℃ are used, a 2-step thermocycling protocol is possible.

Thermocycling Conditions for a Routine 2-Step PCR:

Temperature	Time	Cycles
95℃[a]	5 min	1
95℃ 68℃	15-30s 1kb/min	30
68℃ 4-10℃	5min ∞	1

11. PCR product:

The PCR products generated using HotStart *Taq* DNA Polymerase contain dA overhangs at the 3´end; therefore the PCR products can be ligated to dT/dU-overhang vectors.