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

Catalog: RK20600
Size: 1,000 U / 5,000 U / 10,000 U
Concentration: 5,000 U/ml
Components:

Taq DNA Polymerase (5,000 U/ml)	RM20310
10X PCR Reaction Buffer, Mg^{2+} plus	RM20101

Product Description

Taq DNA Polymerase is a thermostable DNA polymerase that possesses a 5'→ 3' polymerase activity and a 5' flap endonuclease activity. *Taq* DNA Polymerase was isolated from a recombinant source and offers robust and reliable reactions. It tolerates a wide range of templates and incorporates dUTP, dITP, and fluorescently-labeled nucleotides.

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 incorporates 10 nmol of dNTPs into acid-insoluble material in 30 minutes at 75°C.

Reaction Conditions:
1X PCR Reaction Buffer, Mg^{2+} plus

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°C

Storage Temperature: -20°C

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°C

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

Reaction setup:
We recommend assembling all reaction components on ice and quickly transferring the reactions to a thermocycler preheated to the denaturation temperature (98°C).

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	1× (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
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. **Incubate in a thermocycler as the**

below program:

Temperature	Time	Cycles
95°C	30s	1
95°C 45-68°C 68°C	15-30s 15-60s 1kb/min	30
68°C 4-10°C	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 *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. *Taq* DNA Polymerase Concentration:

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

6. Denaturation:

An initial denaturation of 30 seconds at 95°C is sufficient for most amplicons from pure DNA templates. For difficult templates such as GC rich sequences, a longer denaturation of 2–4 minutes at 95°C is recommended prior to PCR cycling to fully denature the template. With colony PCR, an initial 5 minutes incubation at 95°C is recommended to lyse cells.

During thermocycling, a 15–30 second denaturation at 95°C is recommended.

7. Annealing:

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

8. Extension:

The recommended extension temperature is 68°C. Extension times are generally 1 minute per kb. A final extension of 5 minutes at 68°C 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°C are used, a 2-step thermocycling protocol is possible.

Thermocycling Conditions for a Routine 2-Step PCR:

Temperature	Time	Cycles
95°C	30s	1
95°C 65-68°C	15-30s 1kb/min	30
65-68°C 4-10°C	5min ∞	1

11. PCR product:

The PCR products generated using *Taq* DNA Polymerase

contain dA overhangs at the 3´ end; therefore, the PCR products can be ligated to dT/dU-overhang vectors.